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July, 1955

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Medical

BIOCHEMISTRY OF SOME TOXIC AGENTS*

I. Present State of Knowledge of Biochemical Lesions Induced by Trivalent Arsenical Poisoning

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These two lectures represent a summary of our attempts to penetrate the biochemical curtain which veils the action of some toxic agents upon the tissues of higher animals, a necessary step towards the more logical use of drugs.[1] It is a particular pleasure to have the opportunity of lecturing here on this subject in the home of scientific medicine.

Most of my work with colleagues in Oxford has been based upon systems of enzymes rather than upon the isolated enzyme. This is not because I think that work on isolated enzymes is unnecessary—in fact it must be an essential step in progress; but I have personally preferred to work mainly upon instances where biochemical changes *in vitro* can be related directly to some obvious pathological state of an animal because this gave me more confidence that the biochemical facts had biological meaning. Though studies on artificial mixtures of enzymes should always contribute to chemistry, it is a possible risk that they may not be applicable to events *in vivo*. It is almost a platitude to say that if we can really understand these changes *in vivo* at the molecular level (what I have called the biochemical lesion), we must inevitably throw some light on pharmacological and pathological events.

Today I am summarising one section of the complex arsenical field, as I see it personally (1). Though I hope that further practical applications may lie ahead I propose today to treat the matter mainly as a scientific problem.

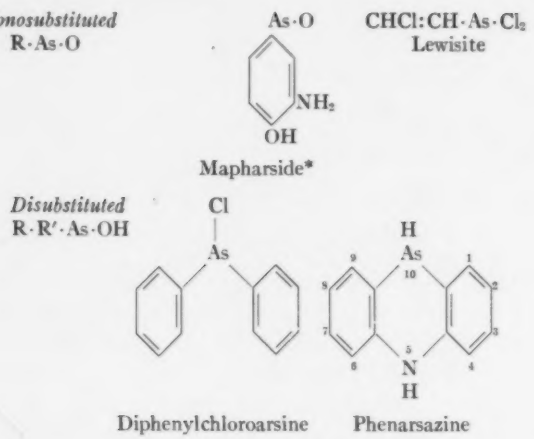
We may refresh our memory of some of the various types of arsenical compounds which are relevant.[2] The trivalent (tervalent) arsenicals are the

* The Charles E. Dohme Memorial Lectureship, Eighteenth Course delivered at the Johns Hopkins Hospital, November 9 and 10, 1954.

[1] Though I initiated this approach earlier, it must now be placed side by side with the antimetabolite competitive approach given so much impetus by the work of Fildes and Woods; the microbiological approach from all levels of biological organisation has been admirably set forth in perspective recently by Woods (2).

[2] *Notes on Nomenclature:* It is usual now to call trivalent, tervalent, so that this term has been used throughout. The use of arseno and arsenoso is still not generally understood and I have not employed these terms. A more doubtful point arises in the nomenclature of the arsenic-sulphur linked compounds; thioarsenite is often used but seems incorrect. I have

FIG. 1. Tervalent arsenicals, (arsenoso)



basic compounds responsible for the toxic action of most arsenical compounds, because arsphenamines now have been shown to be converted even by traces of oxygen to the tervalent form. This type of approach was foreshadowed by Ehrlich and definitely proved by Voegtlin and Smith (3) (4).

It is a truism that the whole problem of arsenicals in relation to arsenotherapy and to toxicity is tremendously complex, involving, as it must, penetration through the cell barriers to the active centres in the tissue and the attempt to get a differential toxicity to the microorganism as distinct from the host. Even when inside the tissue itself we now know that an arsenical must pass at least one other permeability barrier to reach the enzymes in the mitochondria; hence any antidote must be capable of extracting it past these "doors." The complexity of the mitochondrial organisation has been wonderfully revealed recently in the electron micrographs from the Rockefeller Institute (Palade) (5) and elsewhere. Some of the problems of permeability are directly connected with ionization as was shown for instance for the acid substituted phenyl arsenoxides (6). Some are more complicated still and involve the development of arsenical resistance, the mechanism of which is still a puzzle.

Whichever way, however, we approach the problem, it reduces itself in its final and most fundamental stage to a biochemical attack by a trivalent

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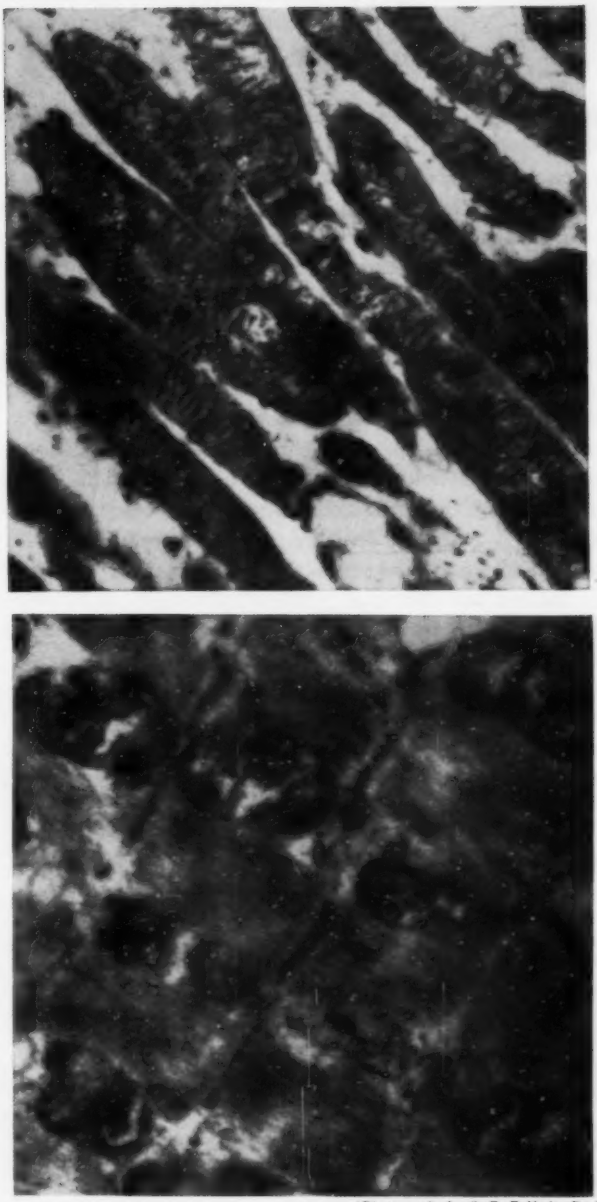
followed the nomenclature of H. King and used thioarsinite, though I realise that this is not rigidly accurate and that a compound such as R—As(OH)(SR) could well be called a thioarsonite.

* Also called arsenoxide, mapharsen, oxophenarsine.



(Photographs by G. E. Paliade, Rockefeller Institute.)

FIG. 2. Electron micrographs of mitochondria (J. Histochemistry and Cytochemistry, 1953, **1:** 188); section cut by the microtome described by A. Claude, Harvey Lectures, 1948, XLIII, p. 152; approximate thickness less than 0.05 μ. The tissues were fixed in buffered osmium tetroxide at pH 7.3 and embedded in polymerized methyl methacrylate. *Top:* rat kidney mitochondria (× 22 850). Note the fine structure, especially the internal ridges (cristae mitochondriales) protruding towards the interior of the organelles. There is a membrane present about 7 mμ (70 A) thick. *Bottom:* rat myocardium (× 22 500).

arsenical upon some component of a cell, so important to it that there is no satisfactory alternative path for the biochemical reactions required for maintaining cell integrity. I have found occasionally that a physiologist finds a philosophical difficulty in accepting the idea that the blocking of one enzyme can wreck a tissue cell; but I think that biochemists could quote a few examples of cases where there is not an alternative path—thiamine deficiency in a tissue using carbohydrate, like brain, is one instance. It is often different in physiology where alternate paths may exist e.g. the circulation. In searching for antidotes we want to find some penetrant compound capable of combining better with the arsenical than this does with the tissue component. To get the most satisfactory arsenotherapy, the process should be reversed; ideally a compound is wanted which will only liberate its As in toxic form inside the microorganism we want to destroy; we should like it to commit suicide. This is a restatement of one of the basic problems of pharmacology at the biochemical level.

It has been in the past, and is still a practice among biochemists to speak rather loosely about the use of arsenicals and of arsenite; in this there is the unconscious assumption that the biochemical action of arsenicals can be classified together. I hope to make it clear that this is not at all true. In fact biochemists could probably make much more use of arsenicals as selective inhibitors or as biochemical dissecting agents if they took more notice of the individuality of the arsenicals.

An examination of the formulae of trivalent arsenicals shows at once that there are two main classes, mono and di-substituted. The action of these might

TABLE I*

Relative Toxicity of Tervalent Arsenical and Antimony Compounds to the Ciliate Protozoa, Colpidium (C) and Glaucoma (G) Death Caused in 3 Minutes

Type	Aromatic	C	G	Aliphatic	C
		Disubstituted			
R_2 As OH	Diphenylchloroarsine	100†	100†	Dimethyl chloroarsine	1–
	Diphenyl arsenious oxide	129	94		
	10-cyanophenoxyarsine	145	117		
	10-chloro-5, 10-dihydrophenarsazine	57	42		
	Diphenylchlorostibine	101	—	Dimethylbromostibine	2
		Monosubstituted			
R. $As(OH)_2$	Phenylarsenious oxide	<5	4	Methyldichloroarsine	<1
	Phenyldichlorostibine	4	—	Ethyldichloroarsine	same

* This table is compiled from selected data of McCleland & Peters as extended by E. Walker, where fuller details will be found.

† Concentrations (100) killing Colpidium were M/180,000 and Glaucoma M/420,000. Colpidium now called Tetrahymena geleii.

be expected on chemical grounds to be different. In 1917, in the hope of introducing some logic into the subject, McCleland and I (7) looked into the relative toxicity of these compounds to ciliates, that is the concentration killing (or immobilising) in 3 mins. Table I shows the results of these simple experiments which were later determined more accurately by E. Walker (8) in my laboratory and shown to apply to another ciliate too. Roughly, the aromatic compounds were 10 to 20 times more toxic than aliphatic arsenicals, and the di-substituted were 5 to 10 times more toxic than the monosubstituted.

The di-substituted compounds like diphenylchloroarsine (DA) are not usually fatally toxic in the higher animal; their main action in man is to produce a most uncomfortable feeling in the throat and nose, best described by saying that it feels like a dreadful cold in the head. The feeling however, disappears. In contrast some of the mono-substituted arsenicals, of which lewisite is an example, are intensely and rapidly toxic. Even within 20 mins. of injection into rats, lewisite produces diarrhoea and great distress. This difference in the behaviour to ciliates and to the mammal of the two classes of compounds poses the problem upon which I am still engaged.

Two decades were occupied in defining a relation of arsenicals to SH compounds in tissues, in determining the toxicity to enzymes and in abortive attempts to reverse the action. This is worth a brief sketch to warn of the patience needed to unravel a pharmacological problem and to impress the need for caution in interpretation. It was many years before I understood that there was an essential difference between the attack made by diphenylchloroarsine (DA) and that by the therapeutic tervalent arsenical mapharsen. In 1923–6 Walker and I found that DA knocked out the nitroprusside reaction for —SH groups given by a 'fixed' —SH in skin.[3] Ehrlich in 1909 had indeed suggested —SH as one of the possible groups on which arsenicals acted; but the dominant interest in the SH field was aroused in 1921 by Hopkins' isolation of the soluble —SH substance glutathione. The main interest in the —SH field in relation to As''' was contributed by Voegtlin and his collaborators from 1923 onwards, working on mapharside, who concentrated at first upon effects in relation to the soluble glutathione. Our own work was independent, but was not published until 1928; however, from 1923 onwards I was convinced that SH groups were connected with arsenical action.

Turning to enzymes, the idea that toxic agents poisoned enzymes arose early; in 1920 for instance, Rona and colleagues (see (1)) found that both DA oxide and phenylarsenoxide were very toxic to urease. Some effects were also found upon oxygen uptake in tissues; e.g. by Voegtlin and by Szent-Gyorgyi, but on the whole the attempts made in the 1920–30 decade to connect arsenical toxicity with enzymes were unconvincing. As we can see now, the efforts were

[3] 'Fixed' is used here in the sense of Hopkins & Dixon to mean an SH attached to protein.

premature, and had to wait for biochemical progress upon the enzymes concerned with respiration and with the reactions providing the necessary energy. Adenosinetriphosphate (ATP) was not then known, and the development of ideas as to "SH" enzymes really started only with Hellerman, Perkins and Clark (9) in 1933—that is enzymes requiring intact SH groups for activity.

During these early days, abortive attempts were made to reverse the action, and so to get an antidote. Voegtlin and colleagues could not *reverse* but were able to *protect* against mapharside with large amounts of glutathione. In my laboratory, Walker temporarily reversed the toxicity of DA to ciliates with monothioethylene glycol ($H \cdot S \cdot CH_2 \cdot CH_2 \cdot OH$). These experiments yielded no antidote; but Cohen, King and Strangeways, (10) proved that this was chemically possible by finding that thioarsinites (compounds of tervalent arsenicals and SH compounds) were dissociable in alkaline solution, as expressed in the equation $R\text{-}As(SR')_2 + H_2O \rightarrow R\text{-}AsO + 2\ R'SH$.

The reversal of lewisite and mapharside was finally resolved by our group (in Oxford) by application of SH ideas and the use of the pyruvate oxidase system, which we had studied in relation to thiamine. As this work is known well, I need only remind my audience of the main points; it was lucky that our attention was then focussed upon lewisite as its dithioarsinite is so remarkably stable.

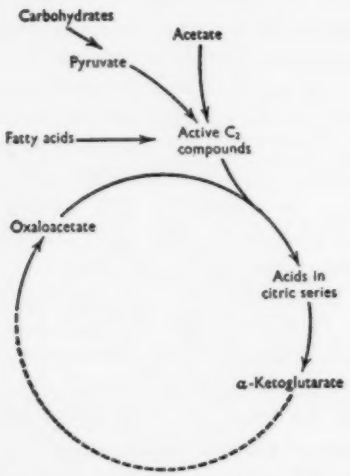


FIG. 3. Diagram of main stages in tricarboxylic acid cycle (see Krebs, 'Advances in Enzymology,' Vol. 3, 1943). Pyruvate, after decarboxylation and activation to a C_2 fragment (now known to be acetyl-coenzyme A (Lipmann)), condenses with the C_4 acid, oxaloacetic acid, to form the C_6 acid, citric acid. By degradation to a C_5 acid and then to C_4 acids, oxaloacetic acid is again regenerated. During the degradation CO_2 and H_2O are eliminated, thereby completing oxidation of the C_2 fragment. The biological reason for these complications is unknown.

Some facts about the system of enzymes in brain tissue oxidising pyruvate are needed. This is now known to include the tricarboxylic acid cycle (11) of Krebs (Fig. 3). The final steps in this argument for brain tissue were provided by ourselves relatively recently (12), (13). The preparations mainly used by us from the pigeon have been 'brei' and ground or 'particle' preparations; the word particle as used by Dr. Lehninger describes the system very well. In both the cells are destroyed, but not the nuclei or the more organized particles, such as the mitochondria; in particle preparations the mitochondria seem more damaged and are less stable than in the brei. With the 'brei', pyruvate is oxidised without reinforcement because the system must still be sufficiently organised to provide its own oxaloacetic acid; the advantage of a brei is that it is relatively stable. The particle preparations require reinforcement with Mg^{++}, adenosinetriphosphate (ATP) and one of the components of the citric acid cycle e.g. fumarate as a source of oxaloacetic acid; in absence of this pyruvate is broken down to acetate, which is not oxidised further. Citric acid itself cannot be used as a source of oxaloacetic acid because the particles are relatively impermeable to it; it was this difficulty which held up our proof of the existence of the citric acid cycle in brain.

In 1949, our group in Oxford, starting upon the ideas mentioned, soon found evidence of the presence of SH in the pyruvate oxidase system by the use of reagents other than As''' (14); we were able to show that the action of lewisite and of phenyl arsenoxide was selective for this system, a smaller concentration of As''' being needed to poison these enzymes than many others. For instance, succinate oxidation in brain brei is not poisoned by lewisite in a concentration giving some 75% inhibition of pyruvate oxidation (15). It was important to decide the most sensitive enzyme because arsenicals in larger amounts attack many enzymes. That pyruvate oxidation was selectively inhibited *in vivo* was proved by showing that pyruvate accumulated in the blood in poisoned animals. (Table II) Later this was confirmed for man (16). In this respect

TABLE II

Effect of Injected Arsenite on the Blood Pyruvate Level of Pigeons

	Mg. pyruvic acid/100 g. blood			Total dose (mg. As_2O_3)
	Normal	Acute	Chronic	
	1.28	4.02 (2 hrs.)	12.39 (4 days)	8.5
	0.94	3.00 (2 hrs.)	14.15 (4 days)	8.5
	0.94	3.12 (2 hrs.)	13.85 (43 days)	7.0
	...	6.30 (3 hrs.)	...	...
Mean	1.06	4.11	13.47	

(From data of Peters, Sinclair and Thompson, 1946.)

Note.—Similar results were obtained using the bisulphite binding method, and with the arsenical lewisite.

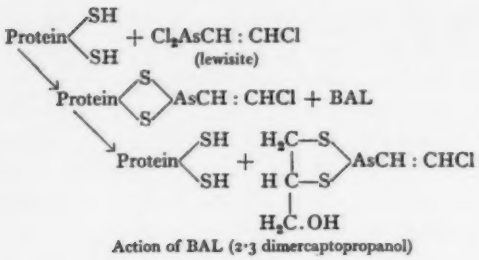
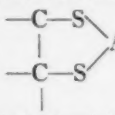

Protein SH SH + Cl₂AsCH : CHCl (lewisite)
Protein S S AsCH : CHCl + BAL
Protein SH SH + H₂C—S H C—S AsCH : CHCl
H₂C.OH
Action of BAL (2·3 dimercaptopropanol)

FIG. 4

arsenical poisoning therefore imitates thiamine deficiency. Our hypothesis as to the enzyme system attacked and as to its possession of an SH seemed correct; but any attempt to reverse lewisite poisoning with glutathione as well as with several other monothiol substances gave completely negative results (H. M. Sinclair), nor could protective effects such as found by Voegtlin et al. in mapharside poisoning be demonstrated.

At this stage there was more than one possibility which could have been followed. However, the final result was reached by two members of our group, Stocken and Thompson; following up Rosenthal's experiment on albumen, they treated kerateine (reduced keratin, Michaelis and Goddard) at neutral pH with excess of lewisite and arsenite, and found that a constant amount of As was taken up even in the presence of excess As;[4] for lewisite this was 0.52%. The As was not taken up after the SH had been oxidised to SS. A large part of the As was combining with 2 SH groups. These experiments led to what we called Stocken and Thompson's dithiol theory; a realization that the special stability might be due to a ring structure, and to the eventual synthesis of 2,3-dimercaptopropanol, named BAL by courtesy of American workers. This vicinal dithiol should form theoretically the most stable 5-membered ring with As, namely  reversed the lewisite poisoning of pyruvate oxidation *in vitro* (Figs. 4 and 5), and acted as an antidote *in vivo*; it was the only way to save an eye contaminated with lewisite, and it actually reversed incipient vesication when rubbed on the arm two hours after an application of lewisite. By removal of As, it frees the action of the enzyme system.[5] It is hardly possible to exaggerate the

[4] The individual differences between the behaviour of monosubstituted As''' showed up even in this chemical experiment, because the arsenite-kerateine compound was much less stable to reprecipitation and to dialysis than the lewisite-kerateine.

[5] This lecture is confined to arsenic; but a reference is needed to the brilliant applications of BAL in Baltimore in the treatment of patients poisoned by mercury, (Longcope and Luetscher, 17).

theoretical importance of this reversal by chemical means of the commencing inhibition of an enzyme system by a poison, as I believe it to be the first instance in which such a possibility has been clearly demonstrated, and in which repair of a tissue by new formation was proved unnecessary.

I must emphasize that none of these effects against lewisite *in vivo* are produced by monothiols; the dithiol is essential to free pyruvate oxidation. It is a qualitative difference. It is remarkable indeed that these effects must be taking place through the two permeability barriers at least of the cell and the mitochondrion.

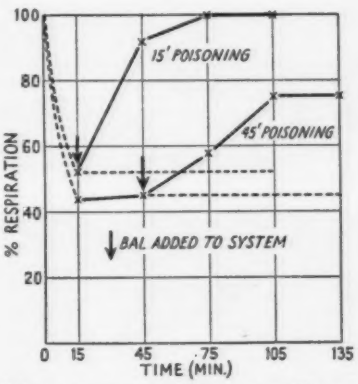


FIG. 5. Reversal of toxicity to pyruvate oxidase system. Pigeon brain brei poisoned with a dose of lewisite oxide sufficient to produce a fall in respiration of about 50 per cent. BAL was added at points indicated after 15 and 45 minutes of respiration. [From data of Stocken and Thompson]

The clinical dividend of this research is well-known; exfoliative arsenical dermatitis due to mapharside has been treated successfully with BAL dissolved in an ampoule mixture devised by Eagle; other forms of arsenical poisoning also respond to this treatment. I will confine myself therefore to some puzzling points. The BAL-mapharside and mapharside-thiolenzyme compounds are more dissociable than the corresponding compounds with lewisite, which can be judged from indirect evidence. NH_2 in the meta position as present in mapharside must decrease stabilities since phenylarsenoxides behave somewhat like lewisite. A higher concentration of mapharside than of lewisite is required to poison the pyruvate oxidase system *in vitro* and rats *in vivo*. Correspondingly, three times the relative amount of BAL is needed for reversal. This explains a curious finding by Stocken and myself in rats; by intramuscular injection in the rat the mapharside-BAL is more toxic than mapharside itself and extra BAL must be added in excess to reverse mapharside-BAL poisoning. This finding has been challenged; but the challenge has been met by Weatherall (18), who has demonstrated that by the intraperitoneal route there is no enhancement

FIG. 6a (*Top*) and 6b (*Bottom*)

FIG. 6. Exfoliative dermatitis, due to arsenical treatment. Fig. 6 a and b, condition at start of BAL therapy; c and d, after 14 days. (Carleton, Peters and Thompson 1948).

FIG. 6c (*Top*) and 6d (*Bottom*)

of toxicity with mapharside-BAL. It is plausible to think that a delay in absorption in the peritoneum in this case gives time for dissociation of the thioarsinite. In treatment of arsenical poisoning due to mapharside, it is evidently necessary to give a sufficient amount of BAL to reverse the enzyme-thioarsinite complex. Dissociability of the thioarsinite in fact allows the successful use of arsenical antidotes.

Lipoic Acid. The results which I have mentioned appeared to be at the time the best proof that lewisite and mapharside attacked SH groups (thiolopoic substances of Bacq) because it was difficult to envisage any other chemical grouping which could be reacting with As in this precise way. One of our research colleagues, V. P. Whittaker, tried to get an idea of the possible size of a ring formed by reaction of the arsenical with the thiols of the enzyme by testing the antidotal reaction *in vitro* of a series of alpha-omega (α-ω) dithiols of the general formula $HS \cdot (CH_2)_n \cdot SH$. As the number of C atoms was increased the antidotal action fell to a minimum with 4 C atoms, which would make with the 2 S atoms and As, a 7 membered ring. It was difficult to picture a small membered ring as part of the protein of the apoenzyme of the pyruvate dehydrogenase, and the problem was left with the suggestion that the ring might have perhaps 5 to 8 C atoms.

During the last few years a final link in this story has come from a series of researches in the U. S. A. all of which have focussed down from various angles upon one compound, variously known as lipoic acid or thioctic acid (Gunsalus, Reed, O'Kane and colleagues; Snell and Broquist; Kidder, Stokstad, Hoffman, Regan; Fordham and Jukes) (19). This compound has been isolated from several sources including liver and yeast, and is an essential component of the pyruvate oxidation system in some bacteria. It is associated with the initial step in decarboxylation. A new compound in Nature, its relation to the trivalent arsenical problem is that it is a 6,8-dithiol octanoic acid. More important still, it has been shown by Gunsalus (19) to be poisoned by arsenite, this poisoning being reversed by BAL and not by the monothiol glutathione.

TABLE III

Inhibition of Apopyruvate Dehydrogenase and Reversal with Dithiols. (Gunsalus, 1953)

Additions	CO_2 Evolution (μl/hr.)		
	No addition	BAL 100 μg	GSH 1 mg
None	26	33	25
α-lipoic acid (10 units)	180	170	184
Arsenite (0.5 mM)	12	170	20

Note: Lipoic acid $H_2C \cdot CH_2 \cdot CH \cdot (CH)_4 \cdot COOH$ (SH, SH)

Reed and de Busk consider that lipoic acid is combined with thiamine.

These researches indicate incidentally that there are still new S compounds to find; in so far as they advance our knowledge of pyruvate and α-ketoglutarate oxidation, they are most important; in the As problem, the finding of lipoic acid solves our difficulties in reconciling the dithiol hypothesis with possible structures in the apoenzyme-protein, as the lipoic acid is a component of the coenzyme. The fact that As will form with lipoic acid a 6-membered ring is also consistent with our evidence, as the stability of this would be less (though not greatly less) than that formed with BAL, and this would explain too the rather slow action in reversing the toxicity of lewisite to the pyruvate oxidase system *in vitro*. There still remains the final step (so far as I know) of showing that lipoic acid is a necessary component of the animal pyruvate oxidase system in brain tissue; but, with this reservation, the detailed action of the monosubstituted trivalent arsenical seems now clear.

Those who have worked upon the action of lewisite and mapharside upon enzymes are now in general agreement that, whereas glutathione or a monothiol will reverse the effect upon most enzymes, the pyruvate oxidase system (and its component α-ketoglutarate dehydrogenase) requires a dithiol; this includes Barron and colleagues (20) as well as ourselves. There is one exception in the literature; Gordon and Quastel (21) claimed that the inhibition of pyruvate oxidation in ground rat brain tissue by mapharside could be reversed by glutathione. As this was a puzzling inconsistency, I have recently reinvestigated the matter, with R. Wakelin. Table IV gives the results. We have found that the poisoning of the residual oxidation is indeed reversed by glutathione; but, upon addition of pyruvate, the extra oxygen uptake so produced is powerfully poisoned by mapharside and not restored by glutathione. The results of Gordon and Quastel could be explained if the grinding had destroyed a large part of the specific pyruvate oxidation.

My final conclusion is, therefore, that monosubstituted arsenicals attack the dithiol (lipoic acid) necessary for the initial stages in the oxidation of pyruvate (and α-ketoglutarate). This poisoning is reversed by a dithiol, but not by a monothiol. The stability of the thioarsinite formed depends upon the structure of the substituent, a meta-NH_2 lowering stability; lewisite or

TABLE IV

Mapharside; Effect of Glutathione on Toxicity to Brain Brei, Rat; O_2 μl 30–90 min.

Note that glutathione restores residual respiration from 13 to 88%, and pyruvate from 4 to 34%. (Peters and Wakelin 1954)

Substrate	None	Pyruvate
No addition	700	2040
+ GSH (50 μmol.)	761	1911
+ Maph. (3 μmol.)	90	75
+ Maph. + GSH	670	645

FIG. 7. Convulsions produced in a pigeon by intracerebral injection of 100 μg lewisite. (Peters & Hastings).

phenyl arsenoxide form a more stable SH compound than mapharside. Reversal depends ultimately upon thioarsinite stability. One final point may be added; since the pyruvate system used in our tests came from the pigeon, it is necessary to show that intracerebral injection of lewisite can cause convulsions.

The behaviour of the disubstituted trivalent arsenicals. Clarification of the biochemical lesion induced by the monosubstituted As''' compounds leaves unsolved the problem mentioned at the start of this lecture. It may be put rhetorically; why is diphenylchloroarsine so much more toxic to ciliates than lewisite or mapharside, and so much less so to the higher animal. Admittedly, this is only a scientific point with little practical application in sight, but I did not feel happy to leave it with no explanation, as these unsolved scientific points are apt to give trouble. Theoretically the diphenylchloroarsine should be capable of attacking enzymes with one SH group to form a monothioarsinite; since the aromatic group enhances toxicity, the monothioarsinite formed with DA should be rather stable. As I have mentioned already, we knew 30 years ago that DA attacked glutathione; but until recently, no example existed of an enzyme which was poisoned by DA at a concentration which left it unscathed by lewisite. Four years ago Dr. Lotspeich working with me found that this was true of isocitric dehydrogenase, a member of the citric acid cycle responsible for the stage isocitric acid $\leftrightarrow$ α-ketoglutarate + CO_2 and requiring TPN. The enzyme was highly reactive to DA, but was untouched by much higher

concentrations of lewisite or arsenite (Fig. 8). More interestingly, the toxicity was reversed by glutathione and monothioethyleneglycol. The effect seemed to be general for disubstituted As‴, because phenarsazines were also toxic though a higher concentration was needed (22).[6] Here, then, was a case in which an enzyme was behaving like the ciliates.

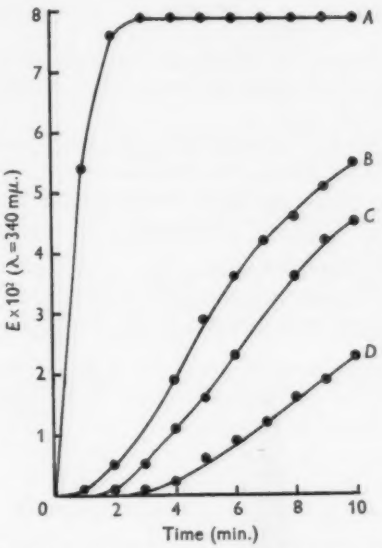


FIG. 8. Inhibition of isocitric dehydrogenase by diphenylchloroarsine (DA) and reactivation by monothiols. Curve A shows the rapid activity of the control in reducing coenzyme II. Curve D—the activity is much inhibited by DA (0.063 mM). Curves B and C—the activity is reversed by glutathione (1.3 mM) or by monothioethyleneglycol (0.63 mM). Note: Lewisite 0.65 mM had no effect. (Lotspeich and Peters, 1951).

Aconitase. Very recently, we have found another enzyme which behaves the same way and which is also a member of the citric acid cycle, viz. aconitase; it precedes isocitric dehydrogenase in the cycle. As I shall be speaking of this also in my second lecture, some remarks are necessary about present knowledge of its function and activation. Its function (Fig. 9) is to establish the equilibrium between the three tricarboxylic acids found in the citric acid cycle. Until lately, this enzyme was so unstable that studies on it were very difficult and attempts at isolation had not been very successful. J. F. Morrison (23) in my laboratory has recently tamed it in the sense that it will remain stable in the cold, and that

[6] As might be expected, the isocitric dehydrogenase was sensitive to Hg compounds, and also reversed by GSH. It was stable to iodoacetate provided that care was taken to remove the last traces of iodine from the iodoacetic acid used.

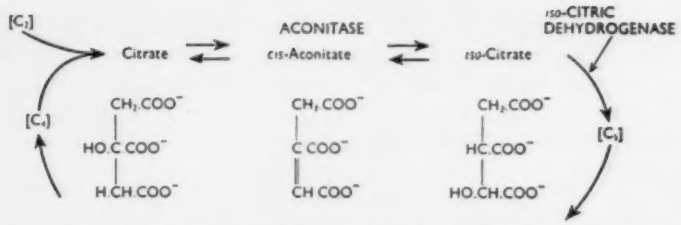
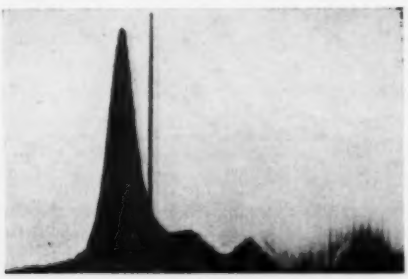


FIG. 9. The reactions of the tricarboxylic acid cycle effected by the enzymes aconitase and isocitric dehydrogenase. At equilibrium about 90% is citrate.

experiments with it are now reproducible. Morrison's work was made possible by the observation that citric acid stabilised the enzyme (Buchanan and Anfinsen (24)), and, more important, that ferrous iron and cysteine were needed for full activity (Dickman and Cloutier 25). Without these the purified enzyme showed virtually no activity.

The final preparation showed one main component in the electrophoresis apparatus (Fig. 10). We found that the action of inhibitors can be studied with



(a)

(b)

FIG. 10. Electrophoretic patterns of the aconitase preparation obtained after fractionation as described in the text. Veronal buffer: pH 8.6; ionic strength, 0.1; temperature, 1°; current, 10 mA; protein concentration, 1%; time, 168 min. (*a*) Descending boundary; (*b*) ascending boundary. (J. F. Morrison, 1954).

TABLE V

Effect of Arsenical Compounds on Aconitase and Reversal by Glutathione

	Conc. (μM)	GSH	Inhibition
1. Arsenite	10,000	nil	0
Lewisite oxide	220	nil	0
Diphenylchloroarsine	57	nil	50%
2. Diphenylchloroarsine	40	nil	70%
DA + GSH	40	2,000	4%

Aconitase was activated by Fe^{2+} and ascorbic acid. Series I. The inhibitors were preincubated with the enzyme for 5 min. in the presence of 0.5 μmol of isocitrate before the addition of cis-aconitate. Citrate formation was determined. Total vol. 3.0 ml, temp. 30°. In series 2, the inhibitor was added at zero time, glutathione (GSH) at + 5 min, and cis-aconitate at + 10 min. A correction was applied for the inhibitory effect of GSH alone in this concentration. J. F. Morrison and R. A. Peters (unpublished results).

confidence provided that the use of phosphate is avoided when substrate is absent and that there is a proper restoration of Fe^{++} and SH compound. Table V shows unpublished results which he has obtained with arsenicals, partly in Oxford and partly in Australia; arsenite and lewisite in relatively high concentration have virtually no effect, whereas diphenylchloroarsine inhibits the enzyme, the inhibition being reversed by glutathione. In these respects the action of the disubstituted arsenical is the same as that for the isocitric dehydrogenase.

Two instances are therefore now available of enzymes which behave to arsenicals as do the ciliates. They provide grounds for a tentative hypothesis in explanation of our scientific puzzle. Since the tissues of the higher animal contain a natural defence in the circulating glutathione, it is likely that any diphenylchloroarsine penetrating skin or mucous layers will form very stable monothiol thioarsinites; as the compound DA-GSH is soluble in aqueous solution, it could even be excreted as such. On the other hand lewisite forms unstable monothioarsinites, which would dissociate readily in presence of tissue dithiols to give the more stable rings with the enzymes containing lipoic acid (Fig. 11). In this way lewisite and mapharside would get under our guard, as it were, and in the absence of BAL become irreversible poisons. We do not know yet the nature of the SH group responsible for the attack made by diphenylchloroarsine upon the sensory nerve endings, an attack which gives rise to tracheal and bronchial irritation; but it may be recalled that lachry-

FIG. 11. Suggested hypothesis:

Arsenical	*Monothiolthioarsinite*	*Dithiolthioarsinite*
Lewisite	Lewisite-GSH	Lewisite-lipoic acid
DA	DA-GSH (excreted ?)	None

TABLE VI

Effect of Diphenylchlorarsine (0.38 μmol) in Reducing Respiration and Citrate Formation by Brain Preparations, and the Influence of Glutathione and BAL in Reducing the Toxicity

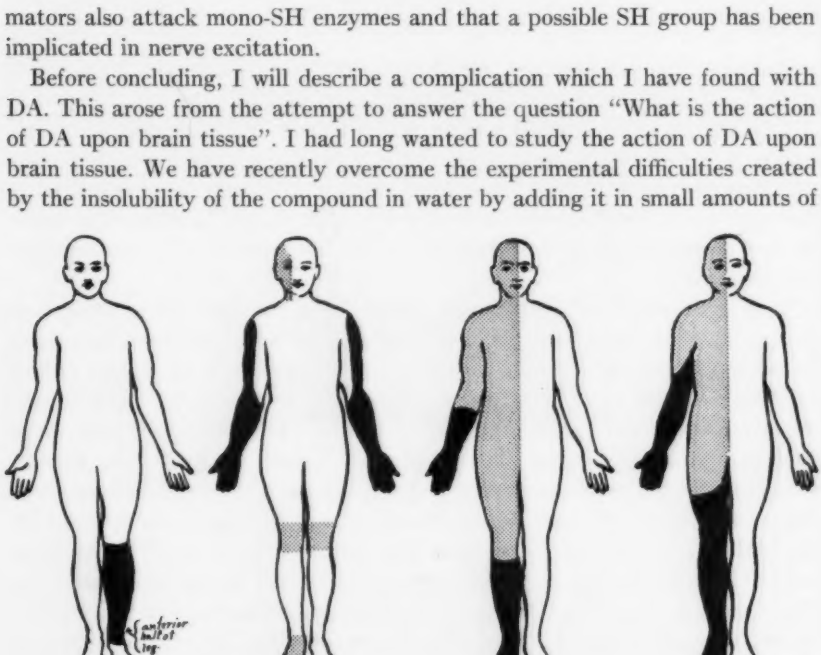
Substrate, pyruvate for brei; supplemented with fumarate and ATP-Mg for particles.
(Peters and Wakelin, 1954)

	Brei O_2	Particles	
		O_2	Citrate
DA alone	−44%	−64%	−88%
DA + GSH	−22%	−30%	−29%
DA + GSH + BAL	−13%	−27%	nil

GSH, brei 50 μmol; particles 25 μmol.
BAL, 0.58 μmol.

mators also attack mono-SH enzymes and that a possible SH group has been implicated in nerve excitation.

Before concluding, I will describe a complication which I have found with DA. This arose from the attempt to answer the question "What is the action of DA upon brain tissue". I had long wanted to study the action of DA upon brain tissue. We have recently overcome the experimental difficulties created by the insolubility of the compound in water by adding it in small amounts of

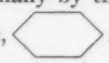


(a) Case 1 (b) Case 2 (c) Case 3 (d) Case 4

Distribution of skin anaesthesia found in four cases of probable chlorarsine gas poisoning. Areas of marked anaesthesia are heavily shaded; areas of less marked anaesthesia are indicated by lighter shading.

FIG. 12. Chlorarsine poisoning, due to so-called arsenical smokes. (From History of the Great War, based on official documents. Medical Services. Diseases of the War. Vol. 2. Edited by Sir W. G. Macpherson *et al.* London H. M. S. O., 1923).

alcohol or as the compound with glutathione. In this way we found in hitherto unpublished experiments that the DA has a toxicity of the same order as that of lewisite. Upon the hypothesis outlined, glutathione should reverse this toxicity. Table VI shows that it only has a partial effect; but the addition of BAL to the GSH much improves respiration and prevents the accumulation of citric acid by the brain particles.

Hence in brain particles (and it applies to kidney particles too) DA makes an attack on an enzyme needing 'dithiol' for reversal as well as a monothiol substance. We may interpret this provisionally by the hypothesis that tissues can convert DA partly to phenylarsenoxide,  $As(OH)_2$. If substantiated, this finding means that therapy of poisoning by disubstituted arsenicals should include glutathione as well as BAL.

Is there any indication that such a scission of the arsenical could occur *in vivo*? The only suggestion in favour of it which is known to me is to be found in an old record of so-called chloroarsine poisoning, reproduced in Fig. 12. The attack upon the central nervous system here could be interpreted as a 'dithiol' attack formed secondarily from the diphenylchloroarsine.

Summarizing this section, I put forward as a hypothesis the view that the action of DA and of other disubstituted arsenicals is fundamentally an attack upon an enzyme needing 'monothiol' for its activity, and that this can be handled largely by the body's own monothiol defences. If some tissues can convert DA to phenylarsenoxide, as they appear to do *in vitro*, then the natural defences would have to be supplemented by BAL as well as by glutathione. In any case it would seem wise to use a dithiol-monothiol mixture in therapy.

In this lecture, I have tried to put in perspective my views of the biochemical lesions induced by the two main classes of trivalent arsenical compounds, and to show that their actions may differ widely in detail; in each case the severity of the attack will depend upon the thioarsinite formed with the enzyme, and the stability of this is influenced by the nature of substituent groups, e.g. a meta-NH_2 lowers the stability of the dithiol thioarsinite.

These are the fundamental points upon which I feel that arsenical therapy must be based, and which I hope will contribute to the analysis of all the further complications (26) inherent in therapy *in vivo*.

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BIOCHEMISTRY OF SOME TOXIC AGENTS

II. Some Recent Work in the Field of Fluoroacetate Compounds

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In my first lecture I have described experiments on the biochemical lesions induced by trivalent arsenical compounds, and have indicated that the most sensitive enzymic point *in vivo* lies in the oxidation of pyruvate. Today I shall be dealing with another instance in which there is a block in pyruvate oxidation, this time in the tricarboxylic acid cycle at the citric acid stage (1).

The substance fluoroacetic acid ($F \cdot CH_2COOH$) has a romantic background. It was first made by Swarts in Ghent in 1896, because he was interested in making C—F compounds. Together with others I was first introduced to it in 1941, because it was thought then that more should be known about it as a defensive measure. No one imagined that Nature had already made it, until Marais in 1943 proved it to be the active principle of the S. African plant poisonous to cattle, known locally as Gifblaar (Pretoria region) and botanically as Dichapetalum cymosum (Fig. 1). The plant has an extensive underground stem system. As little as 20 gm. of fresh leaves may kill a sheep. When given to the rat in a dose of about 5 mg./K, after an interval of 20′ or more, violent toxic convulsions or fits ensue (Fig. 2). The attack on animals is mainly concentrated on either the central nervous system or the heart, and the dose required to kill is very variable; the dog may be killed by a dose $\frac{1}{100}$ of that killing the rat, whereas the toad stands a dose 100 times greater. Man, like the monkey, is probably less sensitive than the rat, as was finally shown by Dr. Adrian on himself about 1941.

Fluoroacetic acid is extremely stable, withstanding for instance hot concentrated H_2SO_4; it cannot therefore act like iodoacetic acid by combination with SH groups. No isolated enzyme is known to be poisoned by it. Tests upon a series of C—F compounds made by Saunders and colleagues at Cambridge, England (2) from 1941 onwards showed that activity seemed to be associated with the presence of a fluoro - C_2 fragment; there was a suggestion that this could be reached by a two-step degradation from longer chain fatty acids because compounds containing an even number of C atoms were toxic, whereas those containing an odd number were much less toxic. Bartlett and Barron (3) thought that fluoroacetate blocked acetate metabolism. During the last seven years, my colleagues and I in Oxford have sunk time in learning more about the toxic agent because (a) we hope to get an antidote for poisoned cattle; (b) it

FIG. 1. Dichapetalum cymosum, (Hook) Engl., showing the underground stem system. From Burtt Davy.

FIG. 2

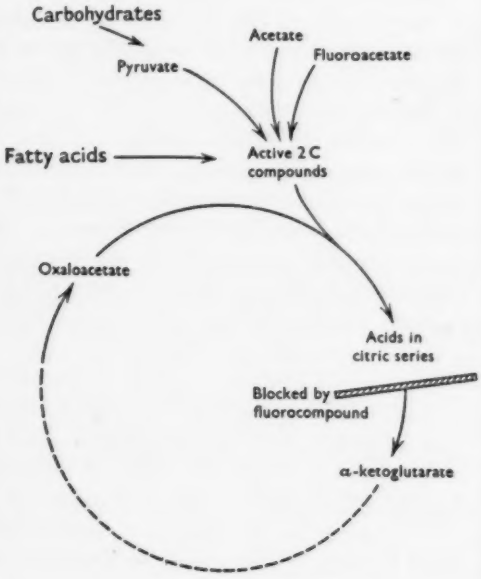


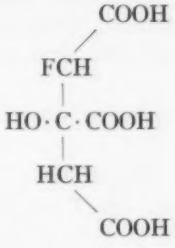
FIG. 3

FIG. 2. Rat in tetanic convulsion after dosing with 10 mg/k fluoroacetate. There is an interval of over 20 min between intraperitoneal injection and convulsions.

FIG. 3. Illustrates the 'jamming' theory of fluoroacetate action. Fluorocitric acid is synthesised via an activated C_2 fragment and then blocks aconitase.

formed a marked exception to the rule that poisons attack enzymes and (c) because of its scientific and probable medical interest, (See Peters, (1)).

Work with Liébecq, Buffa and Wakelin has shown that fluoroacetate caused citrate accumulation *in vitro* and also *in vivo*. This was due to a "lethal synthesis" by which the non-toxic compound fluoroacetate was converted to fluorocitrate by enzymic synthesis from the FC_2 fragment and C_4-dicarboxylic acids. The synthesized fluorocitrate blocks aconitase, so causing a 'jamming' of intermediary metabolism at the citric acid stage, and thereby leading to citric acid accumulation in the tissues (Fig. 3); liver and blood are an exception. In proof of this, fluorocitric acid



was isolated in small amounts as a product of synthesis by kidney particles respiring in fumarate-fluoroacetate mixtures suitably reinforced with Mg^{++} and adenosinetriphosphate (ATP) (4). Fluorocitrate is very active; 1.2 μg. may inhibit the disappearance of 2 μmoles citric acid in 30 minutes in our test system of kidney particles to which 10 μmoles citrate are added initially. Fluorocitric acid has also been synthesized by Rivett (5), via the ester of fluorooxaloacetic acid and bromoacetic ester. The fact that fluorocitrate inhibited citric acid metabolism showed that poisoning by fluoroacetate was not actually an exception to the idea that poisons exerted their effects upon enzymes.

Our most recent work has been concentrated upon the exact mode of action of fluorocitrate on aconitase, upon the convulsive phenomena, upon the problem of the toxicity of another plant, and finally upon the behaviour of fluoro-substances related to the citric acid cycle and upon the details of the mechanism of synthesis.

Before embarking upon this, I should explain more about the nature of the *in vitro* tests which we do with particle preparations made from kidney or brains. After suitable fragmentation in a mortar and centrifuging, the particles are suspended in KCl-phosphate reinforced with Mg^{2+} and ATP, and used in samples corresponding approximately to an original 150 mg. of kidney tissue. When fumarate is used as substrate, enzymatic formation of fluorocitrate can be detected. When citrate is used as substrate, these systems can be used for quantitative determination of fluorocitrate.

FLUOROCITRATE AND EFFECTS UPON ACONITASE

Chemistry. As soon as fluorocitrate became available by synthesis, estimation by the kidney particle test showed that it had roughly half the activity of the enzymic (natural) fluorocitrate.

This is reasonable, because with two optically active centres there should be four isomers; if one centre only matters to the biological activity, then the synthetic should have half the activity of the natural compound. The infra-red spectra of the natural and synthetic compounds were found to be identical by L. C. Thomas (6); it seemed worth deciding chemically whether there was a difference in rotation. This has been done (Peters and Wakelin, unpublished) using molybdate in half the strength usually used. We got for $[\alpha]_D$, L-malic acid as control $+725°$, enzymatically formed fluorocitrate $-314°$, synthetic fluorocitrate, 0.

This clinched the chemical difference between the natural and synthetic compounds.

Aconitase. We now come to the central point of the theory, the attack upon aconitase. We have not found that fluorocitric acid attacks the isocitric dehydrogenase or any other enzyme of the 'cycle'. With Morrison's soluble aconitase which I have described in my last lecture, we have proved finally that natural fluorocitrate is a competitor inhibitor of aconitase, thereby substantiating earlier results with Lotspeich and Wilson (7). The clearest proof of this is the reversal on adding more substrate, which is shown in Fig. 4. All this is

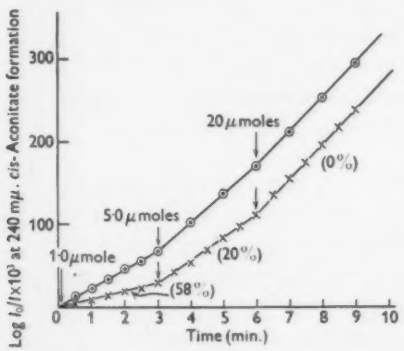


FIG. 4. Inhibition of aconitase by natural fluorocitrate and reversal of the inhibition by increasing concentrations of *iso*citrate. The reaction rates were determined in the presence of 0.05 M phosphate buffer, pH 7.7, as described in the text, using 5 μg. of the final aconitase preparation activated by Fe^{2+} and cysteine. Before the first addition of substrate the enzyme was incubated in the presence of 0.5 μmole of *iso*citrate ± fluorocitrate for 15 min. Total volume 3.0 ml., temp. 22°. ○, Control; ×, fluorocitrate (1.6×10^{-4} M). Arrows indicate the time at which substrate was added. Figures in brackets show the degree of inhibition. Morrison and Peters, 1954.

according to theory. But there are most interesting difficulties in interpretation of the further detail; these start with the fact that the amount used as shown in fig. 4 was 100 μg., i.e. 20 times greater than would produce a large effect on particles. I have interpreted this to mean that the fluorocitrate accumulates relative to the tricarboxylic acids inside the mitochondrial "box".

However the greatest surprise occurred with the synthetic fluorocitrate; though only half as active when using the more organized particles, it was very much more active on the soluble aconitase—roughly speaking the tables were turned (Fig. 5). Furthermore, when the enzyme was preincubated with the synthetic fluorocitrate, there was not complete reversibility on increasing the substrate concentration. This is a chemical point which will have to be cleared

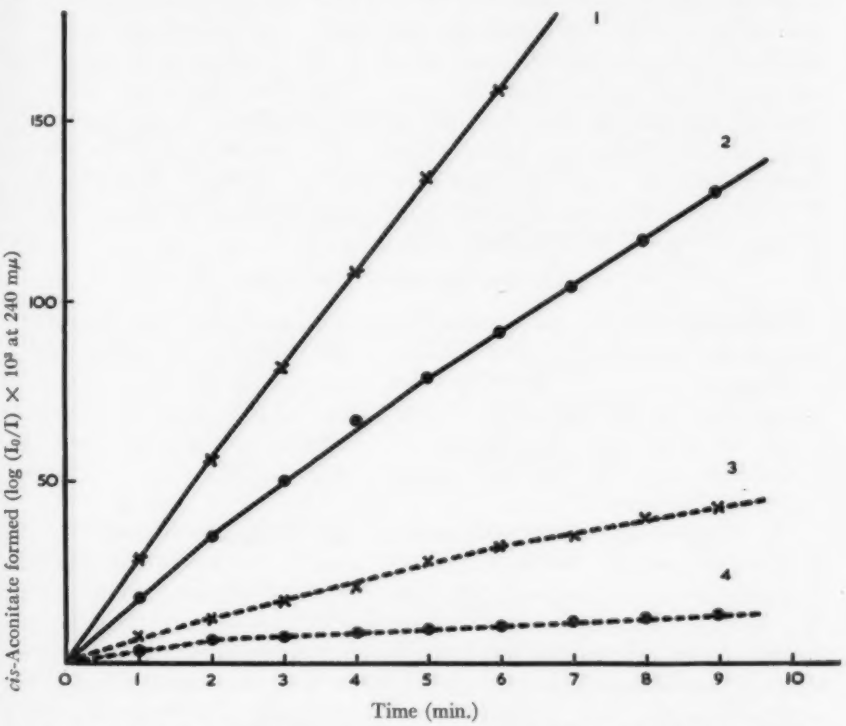


FIG. 5. Inhibition of aconitase by synthetic fluorocitrate. The final aconitase preparation (5 μg.), activated by Fe^{2+} and cysteine, was incubated with fluorocitrate (2.4×10^{-5} M) for 15 min. in the presence of 0.05 M phosphate buffer, pH 7.7, and 0.5 μmole of *iso*citrate. Substrate was then added and the reaction rates were determined as described in the text. Total volume 3.0 ml., temp. 22°. ×, reaction rate in the presence of 16 μmoles of *iso*citrate alone (curve 1); with fluorocitrate (curve 3); ●, reaction rate in the presence of 2 μmoles of *iso*citrate alone (curve 2) and with fluorocitrate (curve 4). Morrison and Peters, 1954.

later. Meanwhile these extraordinary differences between the effects of the unnatural and natural isomers require an explanation. There is no evidence that the mitochondrial particle changes fluorocitrate into some other compound.

Looking at Palade's figures again (8), there are two alternatives; either the mitochondrion is more permeable to one isomer than to the other, which seems unreasonable. Alternatively, we may be driven to the view that this is a case where there is a difference in the orientation of an enzyme inside the particle and in solution. Such a view is not inconceivable; but it would carry with it the awkward implication for biochemists that results with isolated enzymes are not necessarily transferable to the enzyme located in the cell. This, if further substantiated, could well be most important also in pharmacology.*

In summary, and confining ourselves to the natural fluorocitrate it seems a reasonable inference that the attack of fluorocitrate is upon aconitase *in vivo*, and that for therapy it should be possible to reverse the effect by increasing the concentration of isocitric or cisaconitic acids inside the mitochondria. I should mention that we have tried to apply this idea practically in our search for antidotes with no success, even by using amides and esters of tricarboxylic acids in the hope of getting better penetration—so that the final step of a practical proof has not yet been achieved. For the moment the cell barrier appears to be the stumbling block.

FLUOROCITRATE AND CONVULSIONS

This brings me to my next point. What proof is there that fluorocitrate is toxic *in vivo*? It certainly acts *in vitro* upon brain particles oxidising pyruvate; by blocking them with added fluorocitrate, it is easy to show that pyruvate oxidation is decreased with an increase of citric acid. Some preliminary results in my laboratory upon the effect of fluorocitrate *in vivo* have been recently extended by Dr. E. M. Gal (from California) who has been working with me during the last year.

Fig. 6 shows the comparative effect of the intraperitoneal injection of synthetic fluorocitrate and of fluoroacetate upon the citric acid accumulation in kidney, liver and brain of male rats. The most striking difference from fluoroacetate is that fluorocitrate produces an effect in liver; from this it can be concluded, and there is other supporting evidence, that there is little if any synthesis of fluoroacetate to fluorocitrate in liver.† Since these intraperitoneal injections were done with 1½ times the equivalent dose of fluoroacetate, they do not prove that fluorocitrate is the active toxic agent.

The real proof came from intracerebral injection. Over two years ago, when

* Dickman and Speyer (9) in work which I did not see before this lecture was given have found a difference in pH optimum between soluble and mitochondrial aconitase.

† The anomalous results sometimes found with females were excluded by using male animals (11), (12).

the synthetic fluorocitrate became available, I found that it rapidly produced convulsions when introduced into the subarachnoid space in the pigeon skull—a technique I had used for thiamine over 22 years ago. Depending on the dose, convulsions develop in from 10–40 minutes, and persist until death, Fig. 7. They may resemble the head retraction of thiamine deficiency; but usually there is more wing movement. A fit is produced even by 11 μg fluorocitric acid. Injections of fluoroacetate in an amount much greater in terms of F than that of the fluorocitrate does nothing, which agrees with the fact that brain brei does not synthesize fluorocitrate *in vitro*. Hence fluorocitric acid is the toxic agent.

At this time I had the good fortune to be joined in Oxford by Dr. Baird Hastings, whose knowledge of the effects of ionic change in intracerebral fluids naturally proved to be invaluable. We have studied these phenomena together, as we feel that a proper understanding of them must throw light on some clinical conditions (10). As it was important to extend these to a mammal and to ensure that there was no injury to the brain tissue, Dr. Hastings worked out a technique for the rabbit using a bore hole and a suitable tube for introduction of fluids into the skull. (Table I.) Experiments showed very clearly that addition of Ca^{++} would not reverse the death due to convulsions; briefly it may be said that the most varied injections of mixtures of inorganic ions, of glutamine, or of 'cycle' members had no antidotal effect.

We further studied myograms of the induction of these fits (Fig. 8) a year

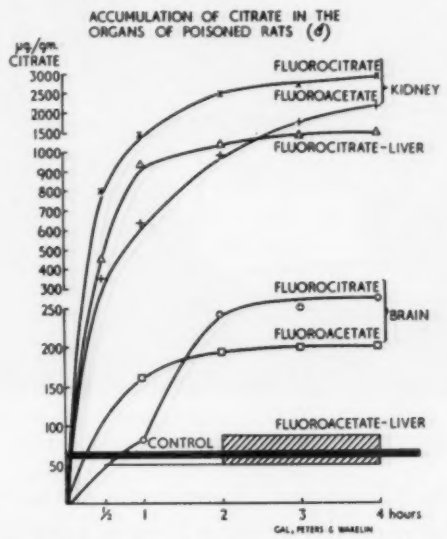


FIG. 6. Comparison of the effects of intraperitoneal injection of fluoroacetate and fluorocitrate. (Gal, Peters and Wakelin, 1954).

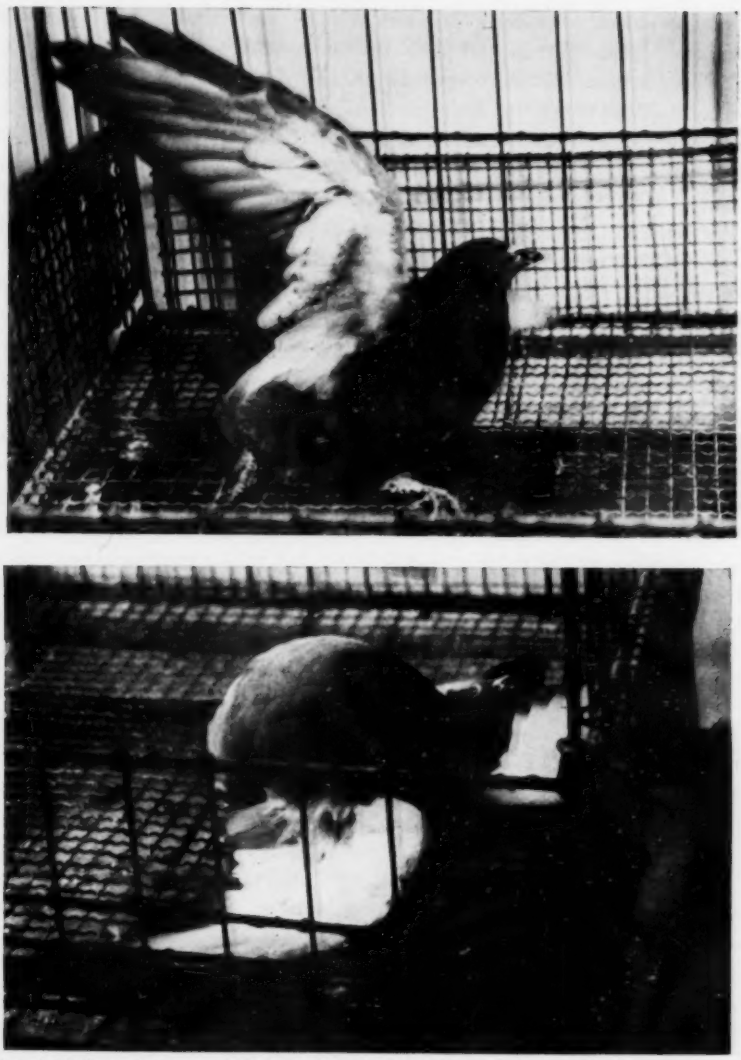


FIG. 7. Effect of intracranial injection (under ether) of fluorocitrate. (a) (*Top*) Convulsion commencing. (b) (*Bottom*) Head retraction.

ago in Boston in the Neurosurgery Department by the kindness of Dr. Sweet. It can be seen that as would be expected the fit is preceded by showers of electrical impulses starting from about 20 mins. after injection and breaking through the nembutal anaesthesia. Dr. B. Hastings showed me about a week

TABLE I

Example of an Experiment by Hastings, Peters and Wakelin upon a Rabbit, November, 1953

Rabbit Anaesthetised with Nembutal

0 min.	Fluorocitrate injection (2 μmoles).
10 min.	Mild signs, head retraction and rapid respiration, arched back.
30 min.	Injected 11 μmoles Ca^{++}. Breathing became regular and legs relaxed, quiet. (This effect was considered to be due to the effect of the citrate ion upon the Ca^{++} in the cerebrospinal fluid.
40 min.	Muscle tremors increasing.
64 min.	General convulsion. Two doses of 8 μmoles Ca were given without effect.
77 min.	Death after convulsions at frequent intervals.

(1) The citric acid content of the brain was raised as has always been found to be the case in fluorocitrate poisoning. Cerebral hemispheres 121 μg/g and rest 212 μg/g.

(2) In the final stages, there was no effect of Ca^{++}.

ago some results for P_{CO_2} obtained by themselves in Boston. These show a very marked alteration as the fit progresses; but the changes in P_{CO_2} preceding the fit are not marked enough to initiate it.

Phosphate esters. To learn more about the convulsive phenomena, Dr. R. M. C. Dawson and I (13) 18 months ago investigated the phosphate ester picture in young rats treated with fluoroacetate and fluorocitrate and plunged into liquid oxygen at the start of convulsion. Table II shows average results. Changes in phosphocreatine were slight and not significantly different for fluorocitrate. Essentially the same results have been published recently by Pschiedt et al (14); an increase in phosphoesters only occurs if the convulsions are allowed to continue. We have also (with Prof. Burn) found no significant changes in acetylcholine.

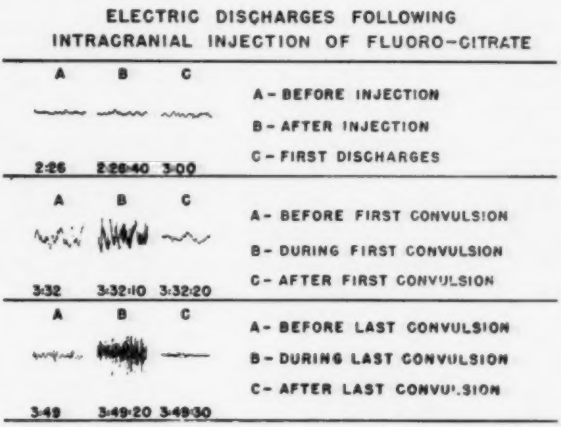


FIG. 8. Electric discharges following intracranial injection of fluorocitrate into a rabbit under nembutal anaesthesia. Time below waves. Hastings and Peters.

TABLE II

Phosphate Ester Determinations in Brains of Young Rats, (32–40 g)

(a) control, (b) injected with intraperitoneal fluoroacetate and (c) injected with intracerebral fluorocitrate. Average for 6 animals. Expressed as μg. per g. brain.

	Calcium Insoluble		Calcium Soluble		Citrate
	P_0	P_{10}	Molybdate-labile P	Res. P	
(a) Control	169	142	95	201	94
(b) Fluoroacetate	187	118	62	205	261
(c) Fluorocitrate	189	117	79	199	198

Note. The change for molybdate-labile P was significant for (b), and not for (c). Changes for P_{10} were barely significant. From data of Dawson and Peters, 1954.

It amounts to this, therefore; interference with aconitase initiates this convulsive state; the metabolism of pyruvate is stopped by jamming of the citric acid cycle and this must give rise primarily to a fall in oxidative phosphorylations. That the slowing of metabolism can precipitate convulsions, we know from the case of thiamine deficiency; but in this instance there is always the extra complication of the citric acid accumulations which cannot fail to immobilise at least some divalent ion. Unless the initiation is so localised that changes in phosphate esters do not show in the general picture as analysed, I incline to the view that the halting metabolism makes some slight ionic alteration in the nervous tissue, and that this induces greater sensitivity to incoming sensory stimuli.

We hope to get more information upon this by using the labelled fluorocitrate which Dr. E. N. Gal has just made in Oxford.

Before proceeding with further biochemical work which we have done upon the pathway of synthesis of the fluorocitrate, I wish to make what must appear an abrupt change but which ultimately fits into our picture. For the last 2½ years at least I have had on my hands the isolation of the active principle of Dichapetalum toxicarium (ratsbane), another species of the same genus as Gifblaar, which grows in the Sierra Leone district of West Africa, and which has been used as a rat poison; the seeds are illustrated in Fig. 9. In 1904 Renner (15) described the effects upon a native of eating some fish poisoned with ratsbane. Briefly, the patient developed a 'neuronitis' e.g. paralysis of the legs and changes in sensation, hyperesthesia, etc.; this cleared off gradually in 3 weeks. He then said that he had often been puzzled by similar signs called "broke back" in the case of young folk and suspected that the local "witch doctors" were responsible. It was rumoured at one time that hardly anyone in the colony died a natural death!

The general signs described for the killing of dogs, etc. from this plant are so similar to those of poisoning by Gifblaar that it was a challenge to prove

FIG. 9. Dichapetalum toxicarium. Photographs of the dry drupe, the nut-like fruit containing the seeds and of the separated seeds.

whether fluoroacetate was also involved; (the C—F bond is a unique rarity anyhow in nature). This was especially important because it would associate a block in the tricarboxylic cycle at the citric acid stage with a clinical condition; one thinks of poliomyelitis and epilepsy. Through the kindness of Sir John Simonsen and others involved, I have studied seeds from Sierra Leone with my assistant Mr. Wakelin. Briefly, we have found that the main toxicity is present in the seed of this "dry drupe"; when ground and mixed with rat cake (a trace of marmite makes it more attractive) 0.5 gm. of the seed may kill a rat, which will die with much fluid about the tissues and large accumulations of citric acid. In contrast a hot water extract of the seeds is not toxic, which should have been the case if it was uncombined fluoroacetic acid as in Dichapetalum cymosum. Thinking that some esterase or other digestive procedure might be necessary to free a fluoroacetyl group, we subjected an aqueous mixture of ground seeds to pancreatin for 24 or more hours. After this the aqueous digest freed of solid matter was given Marais' treatment for the isolation from the mixture of fluoroacetate as the K salt. These methods were partially successful in that a few mgms. of undoubted crystalline K fluoroacetate was isolated in impure state.

Though these experiments established the presence of some fluoroacetate,

TABLE III

Some Examples of Citric Acid Accumulations After Injection in Rats of Seed Extracts from Dichap. Tox. ($\mu g/g.$)

Substance	Brain	Liver	Heart	Kidney
Ground seeds	826	212	3790	412
Fatty acids from seeds	—	—	1280	700
	—	—	1520	625

Note. With fluoroacetate the citric acid accumulation in the kidney is usually greater than in the heart. Data of Peters and Wakelin.

the amount of this as assessed by our methods seemed low, and could not account for the whole toxicity. We eventually found that fatty material, extracted by petrol ether, was toxic *in vivo* in rats and *in vitro* in our kidney particle test. After saponification and fractionation between petroleum ether and KOH, and some further definition in alcoholic solvents, the K salts of the substances were toxic to the rat by intraperitoneal injection in doses of about 20 mg. Rats looked mopey after 5 hours and had specially high citric acid accumulations in the heart as compared with the kidney (Table III).

An inspection of this clinical picture should have told us at once that we were not dealing with fluoroacetate, as it is one that I now know from current experiments to be characteristic of fluoro-fatty acids.

At this stage we had to know whether F was present in these fatty acids. The most reliable and sensitive method for determining F was introduced at Atomic Energy Research Establishment, Harwell, (16); using a hollow cathode source, F is determined in the two F lines, 7128.0A and 7202.4A. After two unsuccessful attempts, a final spectrochemical estimation by Mr. F. T. Birks gave a value of 0.14% F in our sample. Calculation showed that if this F belonged to a K salt of a long chain acid, there could only be 4% of this present in our mixture.

Hence we turned to more powerful methods of fractionation and consulted Dr. A. J. P. Martin of the National Institute for Medical Research (17). He and his assistant, Miss Joan Webb, have fractionated our fatty acids using

TABLE IV

Citric Acid Accumulations (μmoles) in Kidney Particles from the Guinea Pig Induced by Various Fractions Obtained by Reversed Phase Partition Chromatography. Tested at a Theoretical Level of 25 $\mu g/3$ ml; substrate fumarate; run for 30 Min. at 38 C.

Control	0.22
Cut 1	0.85
Cut 2	5.95
Cut 3	0.71
Cut 4	0.30
Rest	0.22

The peak at Cut 2 is reasonably sharp.

reversed-phase partition chromatography, on a 20 cm. column containing Hyflo Supercel treated with dimethyldichlorosilane and liq. paraffin. Aqueous acetone (55%) ran through this. Cuts from the column neutralised with KOH were tested by us on kidney particles with fumarate as substrate and showed remarkably sharp fractionation of citrate-producing activity (Table IV). The peak for the active fraction in one experiment is shown in Fig. 10. These tests were done with a theoretical 25γ of fatty acid, later it was found that as little as 2.5γ could produce accumulations of 4.5 μmoles of cit. from fumarate; even 0.6γ could be detected. This is a much better inhibition than can be obtained with 25 μg of fluoroacetate. I now know that this is a characteristic for fluorofatty acids, as we have obtained it with fluoro-decanoic acid. In regard to the nature of the active fatty acid component separated, a single estimation by Mr. Birk gave the F content as 5.8%, a considerable increase on that in the mixture. You will note that the acid ran somewhat in the position of a 13-carbon acid. The behaviour of two ω-fluorofatty acids kindly sent by Dr. Pattison of London (Ont.) was investigated. They ran about 3 C units faster on the column than the ordinary fatty acids with the same length of carbon chain.

If there be no branching and if the fraction is reasonably pure, which are big assumptions at this stage, a guess would be that we might be dealing with C_{16} fluorofatty acid; but it is no more than a speculation. What seems to be certain is that one of the main components which is toxic in these Dichapetalum toxicarium seeds is some kind of fluorofatty acid; this is therefore a second example of a C—F compound in nature. It is curious that the main activity

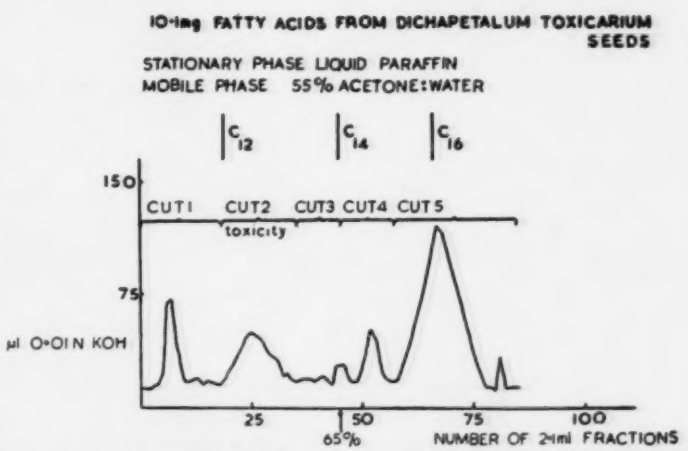


FIG. 10. Fractionation of seed fats by reversed phase partition chromatography. The active fraction separates approximately in the position of a C_{13} acid.

turns up in only one part of the chromatogram; since small amounts of fluoroacetate were also found after a digestion, it suggests that a fluorofatty acid is synthesized from the fluoroC_2 fragments. I have found that the toxic activation with kidney particles involves a synthesis as in the case of fluoroacetate, presumably to fluorocitric acid. Its ready activation by kidney tissue could be explained if it happened to be degraded by the recently found system of fatty acid enzymes, (Lehninger, 18; Lynen & Ochoa, 19; Green, 20).

Previous to this isolation, some work was done on fluorohexanoic and fluorobutyric acids by Barron and Kalnitsky (21), by Kandel and Chenoweth (22) (23). The implication of their work is that a C_2 fragment such as is supplied by monoacetin does not antagonise the action of fluorobutryate for which monobutyrin is necessary. Good as monoacetin is therefore as a protective agent for fluoroacetate, it evidently should not be effective against the Dichapetalum toxicarium fatty acid.

THE SYNTHESIS OF FLUOROCITRATE FROM FLUOROACETATE

In the hope of shedding light upon the synthesis of fluorocitric acid and perhaps of getting some further help towards antidotes, we have been also studying the detail of synthesis of fluoroacetate and of interference with this by acetate. The matter takes one more deeply into biochemical detail; but it is

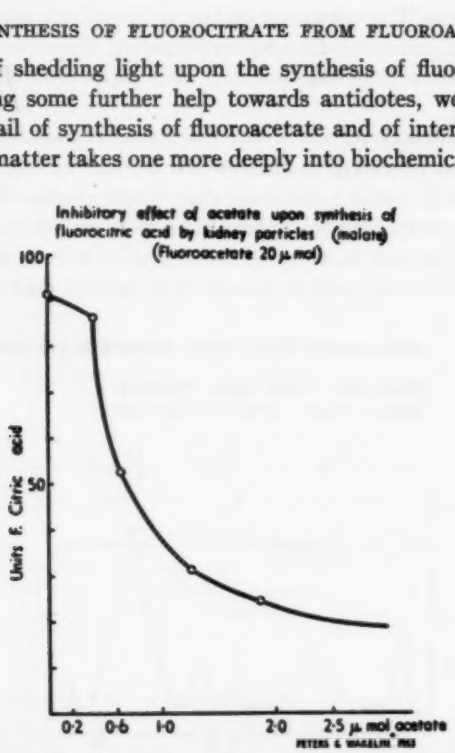


FIG. 11. Inhibitory effect of acetate upon the synthesis of fluorocitric acid by kidney particles as determined with kidney particles; substrate, malate and fluoroacetate 20 μmol/3ml. Data of Peters and Wakelin, 1954.

detail which may become very important and is even now significant, I feel, for biochemistry.

Our first hypothesis for the synthesis of fluorocitrate from fluoroacetate was naturally the simple one that each was activated upon the same set of enzymes, the condensation to citric and to fluorocitric taking place upon the same condensing enzyme. If this be so, then acetate should interfere with the synthesis of fluorocitrate *in vitro* as well as *in vivo*. As any amounts of fluorocitrate formed are small, estimations must be made indirectly with enzymes. Some preliminary tests made two years ago with our kidney particles showed such an interference (Peters, (1) 1952); since then we have confirmed this with much more extensive controls. I can therefore now state that we have proved this in two different ways. Briefly, in one of these we have tested a supposed synthesis by kidney particles (a) upon another kidney particle system using citric acid as substrate and blocking with malonate to avoid re-cycling; in the other (b) we have used the brain particles (pigeon) to test for any fluorocitrate formed. The latter test is not so quantitative; but it is more satisfactory qualitatively because brain tissue cannot synthesize any fluorocitrate from the traces of fluoroacetate still left from the kidney synthesis. As Figs. 11 and 12 show, both these methods indicate that acetate in relatively small amounts interferes biochemically with the synthesis of fluorocitrate from fluoroacetate. This is consistent with the simple idea of one centre for activation and is certainly biochemical support for the mode by which monoacetin protects animals

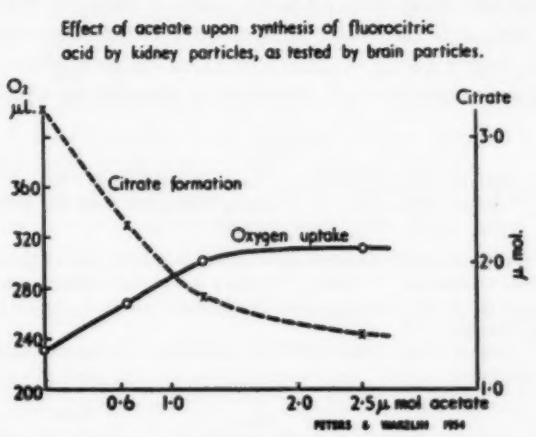


FIG. 12. Inhibitory effect of acetate upon synthesis of fluorocitric acid by kidney particles as tested by brain particles. In absence of acetate, enough fluorocitric acid was formed to give a citric acid accumulation in the test period with brain of over 3 µmol per bottle, with a corresponding depression of O_2 uptake. The presence of 1.0 µ mol of acetate does much to reverse this. Data of Peters and Wakelin, 1954.

from fluoroacetate (Chenoweth, Kandel et al. (24)). The acetyl fragments will stop the synthesis of fluorocitrate.

Unfortunately, the simple hypothesis must be modified, if we are to explain all the phenomena at present known. These can be summarized:

1. The acetone pigeon liver preparation (Stern-Ochoa), as we could confirm, readily synthesizes citrate from oxaloacetate + acetate (in presence of cysteine); it does not make detectable amounts of fluorocitrate when fluoroacetate is substituted for acetate. This means that acetate-active centres exist which will not activate fluoroacetate.

2. All tests by ourselves and others with isolated acetate-activating preparations, using hydroxylamine, as in work on CoA, have given no evidence of formation of fluoroacetyl hydroxamate. This negative result is not due to the failure of any formed hydroxamic acid to react with the iron reagent, because Dr. E. M. Gal has made fluoroacetyl hydroxamate, and has shown that it will give the necessary reactions. Again, known acetate-activating centres do not appear to be activating fluoroacetate.

3. Thirdly, fluoroacetate does not condense preferentially with oxaloacetate. Our kidney particles perform properly in giving citrate from oxaloacetate + pyruvate; this is shown best by using a fluorocitrate block to stop oxidation of the citric acid formed. Yet I have had indication for some time that L-malate was better than oxaloacetate as a substrate for the synthesis of fluorocitrate (Peters, 1952). The point was so important that I have tested it very recently again (with Wakelin) using brain particles. Table V shows by this new method of test that l-malate is the better substrate for fluorocitrate synthesis. This experiment may prove a teaser to biochemists, as the most obvious interpretation is that there is more than one 'condensing' enzyme, by which I mean the

TABLE V

Comparison of the Synthesis by Kidney Particles of Fluorocitric Acid from Fluoroacetate Together with Oxaloacetate or Malate Respectively. The Resulting Fluorocitric Acid was Estimated with Brain Particles

It will be seen that there is little difference in the amounts of citric acid itself synthesised by the kidney particles from oxaloacetate or malate; but there is a marked difference in the fluorocitric acid formed as judged by the decreased O_2 uptake and increased citrate formation in the brain test. Data of Peters and Wakelin, 1954.

Comparision of synthesis of fluorocitric acid from oxaloacetate and malate by kidney particles. Tested with brain particles.

	Kidney, Citrate Found μmoles	Brain			Units Fat
		O_2 μl	Citrate μmoles	Diff.	
Oxaloacetate alone	0.16	—	1.09	—	—
Oxaloac. + fluoroacetate	7.05	−8%	2.09	1.00	30
Malate + fluoroacetate	7.40	−26%	3.21	2.12	150

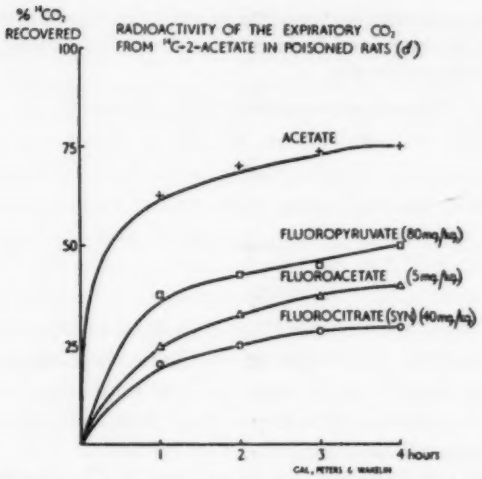


FIG. 13. Radioactivity of the expiratory CO_2 from ^{14}C-2-acetate in poisoned rats, male. Diagram shows also the effect of fluoropyruvate. Gal, Peters and Wakelin, 1954.

enzyme making citrate from acetyl—S—CoA and oxaloacetate. Again an independent pathway is indicated for fluoroacetate.

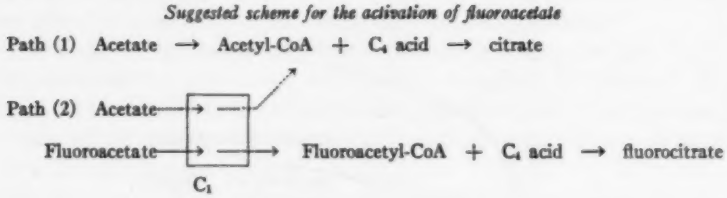
4. Fourthly, there is the point already mentioned that fatty acids other than acetate are needed to antagonise the toxicity of the lower fluorofatty acids (see also (25)). This again indicates that fluorocitrate can be formed by the FC_2 fragments from these fatty acids on some centre in which any FC_2 formed from acetate does not take part. In support of this we have found that *in vitro* butyrate (unlike acetate) has little effect on fluorocitrate synthesis from fluoroacetate. It is to be noted further that our kidney particles in fact activate long chain fluorofatty acids better than fluoroacetate itself.

All this biochemical detail adds up to the fact that at some point in the pathway of the enzymic synthesis of fluorocitrate, there must be activation centres, which are independent of the acetate activating centres and of the fatty acid activating centres.

Even *in vivo*, there are indications that acetate and fluoroacetate are not activated alike. Dr. Gal recently in Oxford injected labelled C^{14} acetate into rats 30 minutes after the injection of fluoroacetate or fluorocitrate, subsequently collecting the CO_2 for 4 hours (26). In Fig. 13, it is shown that with acetate alone 75% was oxidised, confirming Gould, Sinex, Rosenberg, Solomon and Hastings (27); with fluoroacetate there was a reduction in the excretion of labelled CO_2 which confirms Lifson and Swanson (28).* With fluorocitrate

* In these experiments 1.5 microcuries was injected for 50 g. animal; this was present in 0.2 ml solution and 250 μg. Na acetate.

FIG. 14. Acetate is activated by two paths, of which that shown here as path (2) has a centre (C_1) common to acetate and fluoroacetate. Path (1) is the path found in the Stern-Ochoa pigeon liver preparation, etc.


Suggested scheme for the activation of fluoroacetate
Path (1) Acetate → Acetyl-CoA + C4 acid → citrate
Path (2) Acetate
Fluoroacetate
Fluoroacetyl-CoA + C4 acid → fluorocitrate
C1

there was a larger effect (if anything) on the output of CO_2. In these male rats fluorocitrate affects liver metabolism at the citrate stage, whereas fluoroacetate does not. The experiment on the whole animal is of course more difficult to interpret, but if the labelled CO_2 is produced mainly in the liver one might think that fluoroacetate would have the bigger effect.

I have tried to put together a diagram (Fig. 14) indicating all this; it is merely a tentative description.

Before concluding I should like to widen the horizon scientifically by mentioning the behaviour of some other compounds related to acids of the citric acid cycle with which we have been working in Oxford, (N. F. Taylor (29) and P. W. Kent). Except where optically active centres were concerned, it might be expected that the 'cycle' enzymes might not mind the presence of the F; in fact, biochemically, the behaviour follows the title of a well known comedy 'You never can tell'. Table VI makes clear that the acidic and stable FC group induces such profound changes that the compounds are not activated enzymically to form the necessary FC_2 group for making fluorocitric acid.

Fluorooxaloacetate (an intermediate in making synthetic fluorocitric acid)

TABLE VI

Average Results for the Citric Acid Content of Brain, Kidney and Liver After the Intraperitoneal Injection of Various Fluoro Compounds. (C = convulsions).

Gal, Peters and Wakelin, 1954

Compound	mg./kg.	Brain	Kidney	Liver	
		Average citrate content μg./gm.			
Fluoroacetate	5.0	180	1000	57	C
Fluoroacetylhydroxamic acid	8.0	51	242	13	C
Fluorocitrate (syn.)	40.0	240	2500	1200	
Fluoromalamide	300.0	46	98	60	
Fluorooxalacetate	150.0	53	344	16	
Fluoropyruvate	80.0	36	42	23	C
Control		39	45	52	

in vivo only affects slightly citric acid metabolism in kidney. Fluoromalate is virtually inactive. Fluoroacetyl hydroxamate induces no citric acid accumulation, though occasionally convulsions may be seen. Fluorolactic acid (Rivett) is without effect *in vivo* or *in vitro* and hence cannot be oxidised by the lactic dehydrogenase.

Fluoropyruvate (30) is the most peculiar of all; we have found that it cuts down CO_2 formation from radioactive acetate, but has no effect upon citric acid metabolism. It inhibits pyruvate metabolism *in vitro* in yeast and brain, but *in vivo* there is no accumulation of pyruvic acid. Drs. S. L. Thomas and Knivett (in unpublished experiments which they have allowed me to quote) tell me that the metabolism of their phthalate-utilising organisms is blocked at the pyruvate stage by fluoropyruvate. Most curious of all in our experience after 4 hours in rats with a large dose convulsions often appear. Clearly fluoropyruvate is metabolised along a different path and the actual biochemical lesion produced must be different.

Concluding remarks: In this lecture I have spoken of the 'lethal synthesis' of fluorocitric acid from the cattle poison fluoroacetic acid and of the biochemical details of its attack on the enzyme aconitase which accounts for the accumulation of citric acid *in vivo* as well as *in vitro*. This detailed biochemical analysis has not produced an antidote yet owing to permeability difficulties in reaching the mitochondrial enzyme. But the enzymic experiments upon the particles explain biochemically the effect of acetate (or acetyl fragments) in protecting animals as they show that small amounts of acetate stop the synthesis of fluorocitric acid. The work has provided biochemists with a new inhibitor stopping metabolism at the citric acid stage, and has also given a proof *in vivo* of the reality of the citric acid cycle. At the same time several curious points have appeared; among these we may mention the special part played by the mitochondrial organisation in changing the activity of the fluorocitric acid to aconitase, and the question whether there is another condensing enzyme. I have spoken also of the new finding of another C—F compound in Nature, apparently a long chain fatty acid, which was obtained from seeds of Dichapetalum toxicarium. As with fluorocitrate there appears to be a lethal synthesis to fluorocitric acid, and this is presumably concerned with the clinical neuronitis induced by the seeds.

This type of lethal synthesis must be distinguished from the toxic conversions occurring with the arsenical drugs, i.e. conversion of arseno to arsenoso compounds, because here a piece of the body's enzymic machinery is used. Lethal synthesis is thus the opposite of the well-known protective synthesis, in which a toxic substance is made innocuous by the body's enzymes. Though I believe that fluoroacetate is the first proven instance of this 'lethal synthesis,'*

* Marshall, Cutting, and Emerson found in 1938 that acetylsulfanilamide is actually more toxic to the mouse than is sulfanilamide itself (J. Am. Med. Assn. 1938, **110:** 252. Since

some have appeared since in the insecticides; and there is the case of the N analogue of guanine, azaguanine, studied by Kidder et al. and now proved by them and in Cambridge to be due to the incorporation of this into ribonucleic acids of the protozoon (31) and of tobacco mosaic virus (32). There must be many more, and they raise various problems. How does living matter protect itself against its varied 'Achilles heels'. Are there more C—F compounds in Nature? All these questions are for future work.

Meanwhile I would offer these two lectures as my contribution to a logical approach to chemotherapy; and in so far as this is an attempt at a biochemical analysis of problems lending themselves to parallel *in vivo-in vitro* analysis it is a contribution to what I called in 1929 Co-ordinative Biochemistry (33), and also to the fundaments of medicine as well as to pharmacology.

Acknowledgement

My thanks are due to Sir J. Simonsen and Dr. Galley for supplies from Sierra Leone, as well as to those who have collected; to Dr. F. L. M. Pattison, London (Ont.) for samples of fluorofatty acids; to Mr. R. Brown for fluoropyruvic acid; to Sir Alexander Todd for discussion on nomenclature; to Mr. Austin and to Mr. Moseley for photography.

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PRODUCTION OF FEVER BY INFLUENZAL VIRUSES

V. Effect of nitrogen mustard (HN2)*

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Previously reported studies (1–4) have shown that in rabbits the intravenous injection of suspensions of influenza and related viruses results in a febrile response lasting several hours. This has been confirmed by others (5–7). The fever-producing property of influenza virus is more closely related to hemagglutinating ability than any other property of the virus particle. Although it was originally thought that the union of virus particles and some special group of host cells might be fundamental in the genesis of this fever (1–3) subsequent studies have not borne this out completely (4, 7). The finding of a fever-producing substance in rabbit granulocytes (8, 9) suggested the possibility that this cell or some substance released from it after injury might play a role in the febrile response of rabbits to influenza viruses. This has been tested by observing the effect of HN2-induced granulocytopenia upon the ability of rabbits to respond with fever to injection of influenza virus.

PROCEDURE

White male rabbits of mixed breed weighing 2.0 to 3.0 kilograms were used. Animals were placed in metal stalls secured by a loose fitting collar, and rectal temperatures were taken with ordinary clinical thermometers at intervals of 30 minutes throughout each experiment. Before use all rabbits had been allowed periods of acclimatization to the stalls for several days and an adequate number of baseline recordings was obtained before injection of virus into the marginal ear vein.

Allantoic fluid suspensions of the ninety-eighth egg passage of the WS strain of influenza A virus were used throughout the study. Fluids aspirated from 12 day old chick embryos which had been infected 40 to 44 hours previously were pooled, sealed in glass ampoules, and stored at minus 60°C. All fluids were cultured in thioglycollate broth and found to be bacteria-free. The hemagglutinin titer of the pooled fluids as determined by a modification of Salk's method (10) was 1:2048. One-half ml. of this suspension of WS virus was used as the fever-producing dose in all experiments.

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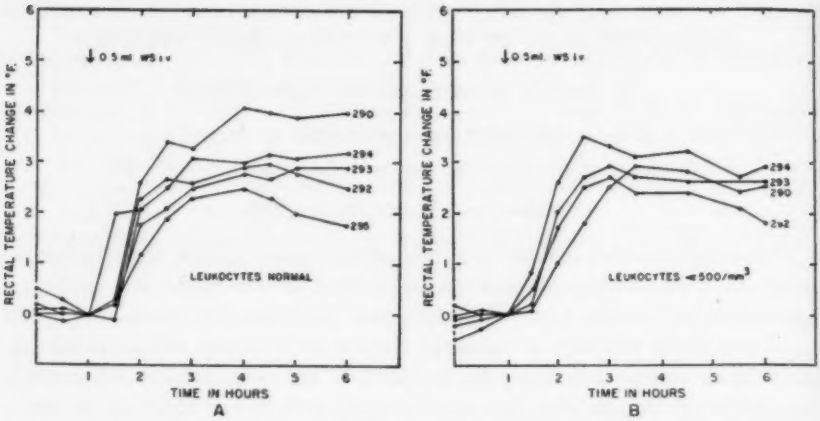

FIG. 1. Fevers after injection of influenza virus in: A. normal rabbits, B. rabbits made severely leukopenic by administration of HN2.

Several rabbits were given HN2 (mechlorethamine HCl, Merck) as a single dose of 5.0 mgm. dissolved in 5.0 ml. of physiological saline injected intravenously. Total and differential leukocyte counts of peripheral blood were carried out prior to injection of HN2 and daily thereafter until leukocytes were reduced to less than 500/mm³, at which time virus was injected and temperatures were recorded.

All needles, syringes, and glassware were sterilized by heating at 170°C. for two hours to avoid contamination by bacterial pyrogens.

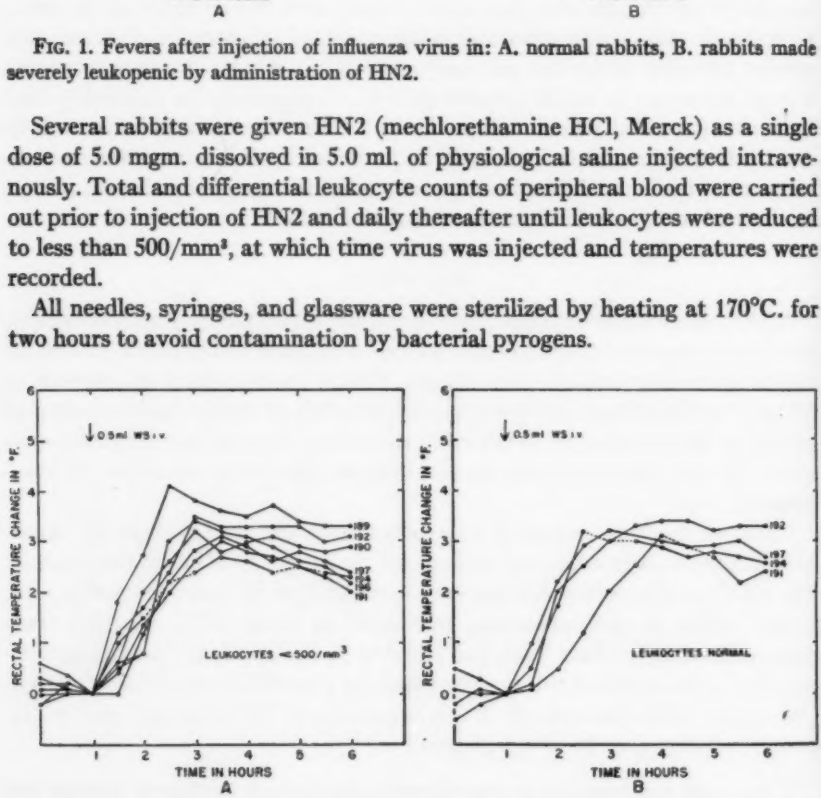

FIG. 2. Fevers after injection of influenza virus in: A. leukopenic rabbits, B. Same animals 18 days later when leukocytes had returned to normal.

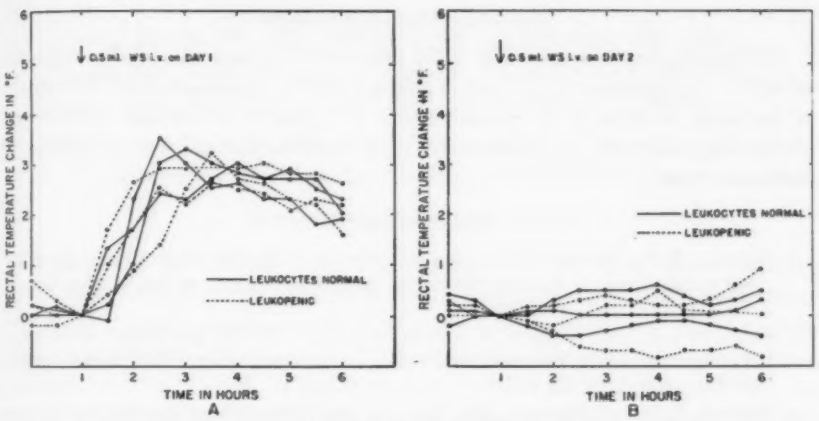

FIG. 3. Temperature curves illustrating the development of "tolerance" to a second injection of influenza virus. Normal or leukopenic animals responded with fever to the virus on day 1 but remained afebrile after the same dose of virus on the following day.

RESULTS

Three experiments were performed. Because a single injection of influenza virus in rabbits produces a state of non-reactivity lasting 10 to 14 days (2), it was necessary to wait for at least this period between fever tests in order to assure that the results were not influenced by this tolerance to the fever-producing effect of the virus.

In the first experiment, five animals were given 0.5 ml. of virus suspension and their fevers were recorded. Eleven days later, all animals received HN2 intravenously. One rabbit died before fever tests were repeated but the remaining four were reinjected with virus on the fourteenth or fifteenth days of the experiment. At this time, their leukocyte counts were less than 500/mm³. As can be seen in Figure 1, the fevers, when these animals were granulocytopenic, were entirely comparable to their original responses.

In the second experiment, seven animals made granulocytopenic by injection of HN2 were tested with influenza virus and were then allowed to recover. The four surviving rabbits were re-tested 18 days later, at which time their leukocytes had returned to normal levels. Figure 2 demonstrates the results of this study. There was no appreciable effect of leukopenia upon febrile responses to influenza virus.

In a third experiment, three leukopenic animals and three normal controls were tested on the same day. All animals responded with characteristic fevers. On the following day, virus injection was repeated and, as shown in Figure 3, all animals demonstrated the expected tolerance (2) to the fever-producing effect of the virus.

SUMMARY AND CONCLUSIONS

Granulocytopenia induced by HN2 failed to influence the febrile response of rabbits to intravenous injection of allantoic fluid suspensions of the WS strain of influenza A virus. It is concluded that the presence of normal numbers of circulating white cells is not necessary for the production of fever in rabbits by influenza virus.

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A STUDY OF THE EFFECT OF THYROXIN ON THE DEVELOPMENT OF TUBULAR DEGENERATION OF THE ADRENAL CORTEX PRODUCED BY ACTH

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It has been pointed out by Rich (1) that in severe acute infections there frequently occurs a type of damage to the adrenal cortical cells characterized by focal necroses of single or several cells, loss of cytoplasm of others, and the accumulation of fluid within the cell columns leading to the formation of tubular structures lined by flattened cortical cells. The lesion was designated as "tubular degeneration" of the adrenal cortex, and when widespread leads to extensive cortical damage. It was pointed out that the peripheral circulatory collapse that characterizes the Waterhouse-Friedrichsen syndrome is frequently associated with this lesion in the absence of the widespread destruction of the cortex by hemorrhage which had been regarded as the essential cause of that syndrome.

Two possible mechanisms require consideration in the attempt to explain the pathogenesis of this peculiar damaging adrenal lesion. On the one hand, the lesion might be a direct result of the action of toxic bacterial products upon the adrenal. On the other hand, since the lesion occurs in the most diverse acute infections, it might result from some bodily alteration associated with the state of infection. Since it is well recognized that the stress of severe infection places an increased demand for cortical hormone upon the adrenals (2), tubular degeneration might be the result of excessive stimulation of the cortex. In order to test the latter possibility, an attempt was made to learn whether excessive adrenal cortical stimulation produced by sterile ACTH could produce the lesion in the absence of infection. It was found that the lesion could be so produced in the rat (3). It is therefore clear that neither toxic bacterial products nor the state of infection is essential for the production of the lesion.

However, the amount of ACTH required to produce the lesion in the rat was very large, and the question arose whether the state of severe infection may entail bodily alterations, in addition to adrenal cortical stimulation, which could potentiate the action of ACTH upon the adrenal. One possible accessory mechanism requiring consideration is increased thyroid activity, particularly since Selye, Stone, Nielsen and Leblond (4) have reported that thyroxin greatly enhances the effect of pituitary extracts in producing en-

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largement of the adrenals. The present experiments were carried out in the attempt to learn whether thyroxin would enhance the action of ACTH in producing the lesion of tubular degeneration.

EXPERIMENTS

Adult male albino Wistar rats were the experimental animals. They were supplied as needed by the Research Supply Company of Philadelphia, Pennsylvania. The animals were allowed to acclimate themselves to the laboratory for several days before each experiment. Tap water and Ralston's Purina laboratory chow were supplied to the animals in separate cages *ad libitum*.

Armour's beef ACTH, a lyophilized sterile powder in individual vials, each containing an amount of ACTH standardized in international units, was used throughout. This ACTH was free from contamination with thyrotropic, somatotropic and gonadotropic hormones, according to Armour Laboratories. One lot was used throughout, except for 4 animals which received Acthar-gel (Armour). The daily amount of ACTH was given subcutaneously in four divided doses, the injection being made every 6 hours around the clock, with a tuberculin syringe and a No. 27 needle. The amount of ACTH required at each injection was freshly dissolved in sterile saline that was introduced into the vials in appropriate amount. When all of a prepared solution was not used at a given injection time, the excess was kept refrigerated until the next injection. No solution was kept more than 6 hours before being used.

In all series the total amount of fluid containing the ACTH or thyroxin to be injected at each time period was deposited subcutaneously at one site, though a different site was used for each injection. The amount of fluid deposited was never more than 0.25 cc. except for the Acthar-gel (Armour) series in which a single injection of 1.25 cc. was injected at one site each day. Preliminary to the injections the hair was shaved and the skin swabbed with 70 per cent alcohol.

The thyroxin crystals† were weighed on an analytical balance and dissolved in sterile saline adjusted to pH 12 with sterile 10 N NaOH. After the thyroxin dissolved, the pH was brought to 8.5 with sterile 10 N HCl. This yielded a flocculant white precipitate which was resuspended by shaking before each injection. The injections of thyroxin were begun 3 days before beginning the ACTH injections, and were continued throughout the experiment. Thyroxin was given in amounts according to body weights that have been found by others (5) (6) to raise the basal metabolic rate of rats to plus 75 per cent. No basal metabolic tests were carried out, but the heart rate of the thyroxinized animals was increased to approximately double the normal‡, their temperatures became elevated 1.5° to 3° F. above normal, and most of them lost appreciably more weight than did those treated with ACTH alone.

The animals were routinely killed by etherization in a glass jar. All animals were killed 6 hours after the last injection and were immediately autopsied. The adrenals were removed with great care after ligating the veins, and were fixed intact. The tissues for routine sections were fixed for 4 hours in Zenker formol, followed by 6 hours fixation in 10 per cent formalin, after which they were washed in running water for 6 hours before being placed in 80 per cent alcohol to begin dehydration for paraffin embedding. Routine sections were made from the adrenals and the injection sites. The other tissues were perserved in 10 per cent formalin.

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† The thyroxin was very kindly provided by E. R. Squibb & Son, New York.

‡ In spite of the rapid heart rate of the rat, the electrocardiographic check, for which I am indebted to Dr. Richard Ross, showed that practice enables one to estimate the rate by palpation with reasonable accuracy.

12.5 and 25 mg. ACTH daily: In our previous study (3) these dosages of ACTH were found to be insufficient to cause tubular degeneration of the adrenals in the rat. The purpose of using these dosages in the present study was to determine whether thyroxin would potentiate the action of ACTH to

TABLE I

Summary of Findings in Thyroxinized Animals

Rat No.	Body Wt. Gms.	Wt. Change Gms.	Daily Mgm. ACTH[1]	Daily Mgm. Thyroxin[2] per 100 Gms. Body Wt.	No. of Days ACTH	Colloid Globules	Necrosis of Individual Cells	Tubular Degeneration	Focal Hemorrhage
212	183	+1	12½	0	3	0	0	0	0
213	178	+8	12½	0	3	0	0	0	0
218	176	−7	12½	0.2	3	0	0	0	0
219	178	−5	12½	0.2	3	0	0	0	0
214	188	+5	25	0	3	0	0	0	0
215	162	−5	25	0	3	0	0	0	0
220	156	−16	25	0.2	3	0	0	0	0
221	188	+6	25	0.2	3	0	0	0	0
216	176	+1	50	0	3	+	+	++	0
217	168	−7	50	0	3	++++	+	++	0
222	150	−25	50	0.2	3	+	0	++	0
223	158	−10	50	0.2	3	0	0	++	0
227	194	0	50[3]	0	3	0	0	+	0
228	224	+14	50[3]	0	3	0	+	+	0
232	178	−16	50[3]	0.2	3	0	+	++	0
233	184	−16	50[3]	0.2	3	0	+	+	+
236	180	−4	50	0	4	++++	+++	++++	++
237	170	−18	50	0	4	+++	+	++++	+
238	185	−3	50	0	4	++	+	++++	0
239	176	−12	50	0	4	++++	+	++++	+
242	160	−20	50	0.2	4	+	+++	+++	++
243	150	−30	50	0.2	4	++	+	+++	+
244	158	−32	50	0.2	4	0	+	++	+
245	162	−30	50	0.2	4	++	+	++	+
226	198	−6	100	0	3	++++	+++	+++	0
234	172	−14	100	0	3	++	++++	+++	+++
235	166	−16	100	0	3	++	++++	+++	+
231	178	−34	100	0.2	3	++	++++	++++	+
240	160	−34	100	0.2	3	+	++++	++++	++
241	170	−20	100	0.2	3	++	++++	++++	++
224	214	+28	0	0	4	0	0	0	0
225	240	+32	0	0	4	0	0	0	0
229	222	+8	0	0.2	3	0	0	0	0
230	208	−2	0	0.2	3	0	0	0	0

[1] The animals listed "0" in this column received the ACTH diluent, only.

[2] The animals listed "0" in this column received the thyroxin diluent, only. The administration of thyroxin was begun 3 days before beginning the injections of ACTH or ACTH diluent.

[3] Acthar-gel (Armour).

the degree of causing tubular degeneration from dosages of ACTH insufficient to produce the lesion in the absence of an excess of thyroxin. The adrenals of all animals receiving the larger dosage of ACTH were slightly enlarged and hyperemic. There was no significant difference in the macroscopic or microscopic appearance of the adrenals in the animals that received thyroxin as compared with those of the animals that received ACTH without thyroxin. As in our previous study, tubular degeneration developed in none of the animals receiving these dosages of ACTH.

50 mgm. ACTH or Acthar-gel daily: The adrenals of all animals treated with this dosage were hyperemic and significantly larger than normal, the glands of the animals treated for 4 days being several times normal in size. The adrenal veins were correspondingly engorged. The animals receiving this dosage for 3 days developed a moderate degree of tubular degeneration of the adrenals. Thyroxin exerted no significant macroscopic or microscopic effect upon the size of the adrenals or the severity of the cortical lesions in this group. In the group of animals that received this dosage of ACTH for 4 days, those that were treated with thyroxin developed rather less severe lesions than those that received ACTH alone.

100 mgm. ACTH daily: The adrenals of all animals receiving this dosage were decidedly enlarged and hyperemic. There was no macroscopic difference between those of the thyroxinized animals and those of the animals that received ACTH only. Microscopically, adrenal tubular degeneration was well developed in all of the animals, those treated with thyroxin showing somewhat more severe lesions than those receiving ACTH alone.

Non-ACTH Controls: After the preliminary 3 days treatment with thyroxin, two animals were given thyroxin and ACTH diluent for 3 days; and after 3 days preliminary treatment with the thyroxin diluent, two were given ACTH diluent and thyroxin diluent for 3 days. No lesions were found in the adrenals of any of these animals.

Table I is a composite of 4 separate experiments. In each experiment except one (Nos. 224, 225, 229, 230) all of the animals received ACTH in the amounts indicated in the Table, and half of them received thyroxin and half thyroxin diluent. The Table lists the occurrence and degree of tubular degeneration and other adrenal cortical lesions which occur in the rat as a result of overstimulation of the adrenals by ACTH (3).

DISCUSSION

The present experiments were carried out in an investigation of the factors concerned in the pathogenesis of the tubular degeneration of the adrenal cortex which can occur rapidly during the stress of fulminant acute infections in man (1), and which we have produced in the rat by the injection of ACTH (3). Since the amount of ACTH necessary to produce the lesion in the normal rat was very large, it was thought that the state of infection might entail some

additional factor which would potentiate the action of the ACTH liberated under the stress of infection. One possible factor requiring investigation was thyroxin, for it has been shown by numerous investigators (7, lit.) that the injection of thyroxin stimulates a marked enlargement of the adrenals; and Selye, Stone, Nielsen and Leblond (4) have reported that thyroxin administered together with crude adrenocorticotropic-containing pituitary extract markedly potentiates the action of the latter in producing enlargement of the adrenal cortex, though Selye (8) states that he made no adequate study of the influence of thyroxin upon the degree of adrenal enlargement produced by purified ACTH.

In both Selye's experiments (4) and those in which thyroxin alone was found to produce adrenal cortical enlargement (7), the thyroxin was administered for periods of several weeks or more. In the present study, which was concerned only with events that may occur during fulminant acute infections, the thyroxin was administered during six or seven days only. At the end of these periods no appreciable difference in size between the adrenals of the thyroxinized animals and their controls was detected visually. The glands were, however, not weighed, for it was found in our previous study (3) that in order best to preserve the histological structure of tubular degeneration in the rat's delicate adrenal, it is advisable to ligate the adrenal veins before removing the glands and to handle them as little as possible before fixation. Ligation of the vein imprisons variable and indeterminate amounts of blood in the adrenal capillaries which, when the gland is stimulated by ACTH, dilate from a moderate to a very marked degree.

In the present experiments no potentiating effect of thyroxin upon the action of purified ACTH in producing tubular degeneration, colloid globules, hemorrhage or necrosis of the adrenal cortex could be demonstrated. The differences in the degree of lesions exhibited by the thyroxinized ACTH-treated animals and their controls were neither consistent nor of a magnitude surpassing the limits of individual differences in response to ACTH alone observed in our previous study (3); nor did thyroxin cause the production of tubular degeneration, or the other adrenal lesions that result from marked overstimulation of the cortex by ACTH, when the thyroxin was administered together with doses of ACTH below those which produce the lesions in the absence of thyroxin. Under the conditions of the present experiments, therefore, no support was obtained for the possibility that an increased liberation of thyroxin during fulminant acute infection may potentiate the action of ACTH in producing tubular degeneration of the adrenal cortex.

SUMMARY

Under the conditions of these experiments, thyroxin exerted no significant effect upon the degree of tubular degeneration of the adrenals that results from excessive stimulation of the adrenal cortex by ACTH. Thyroxin neither

increased significantly the severity of the tubular degeneration produced by an effective dosage of ACTH, nor did it cause the lesion to appear when administered together with amounts of ACTH that were insufficient to produce the lesion in the absence of thyroxin.

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SEVERE MYOTONIA AS A COMPLICATION OF POST-OPERATIVE THYROID DEFICIENCY

Complete Relief of Myotonia by Thyroid Extract. Report of Two Cases

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Myotonia is a standard symptom in *myotonic dystrophy* and in *myotonia* congenita, or Thomsen's disease. *Paramyotonia* is also described. In this condition the myotonia occurs only when the patient is exposed to cold. All of these conditions are inherited.

Occasional cases of *myotonia acquisita* are also described and a number of articles have been published in which evidence is presented to show that thyroid deficiency plays an important role in some of these cases. Two syndromes are described. In 1892 Kocher (6) stated that in sporadic cretinism and in cachexia strumaprivia there might be slowing of the muscular contraction and enlargement of the muscles. A series of articles confirming these conclusions have appeared since then. In 1935 Debré and Semelaigne (3) described two cases of this type in children and this syndrome has since been known by their names. In such cases there is thyroid deficiency, enlargement and abnormal firmness of the muscles and slowing of muscular contraction. If thyroid extract is administered, the muscular disorders disappear. There is no myotonia. This condition is found most frequently in children.

In 1897, Hoffman (5) found evidences of myotonia in addition to the signs and symptoms mentioned above in a patient suffering from thyroid deficiency. Some eighteen cases have been reported since then. Some of these occurred in childhood and some in adult life. The more recent articles are those of Mollaret and Sigwald (10) in 1939, Maas and Paterson (8) in 1939, Hesser (4) in 1940, Lengre and Huguenin (7) in 1941, Theibaut and Henrot (14) in 1943, Coste (2) in 1943 and Alajouanine (1) in 1948. In these cases the picture of myotonia was not always complete. Nevertheless, it is stated that the relaxation of the grip was delayed in some instances, percussion myotonia was sometimes present and a prolonged contraction following faradic stimulation and galvanotonus were both observed. The symptoms were increased by cold and diminished by warmth. The muscles were usually large and unduly firm but were sometimes of normal size and consistency. Muscle cramps were mentioned several times. Adequate treatment with thyroid extract gave prompt relief. Tetany played no role in this condition and when determinations of the serum

calcium content were made they were always within normal limits. This condition has been termed *Hoffman's syndrome.*

Thomasen (15) in his authoritative monograph, "Myotonia, Thomsen's Disease, Paramyotonia, and Dystrophia Myotonica", analyses all of the case reports mentioned above and concludes that true myotonia is probably not a complication of thyroid deficiency. It should be pointed out that Thomasen had an opportunity to examine only one patient who gave a history of myotonia and thyroid deficiency. When the examination was made, the patient had been taking thyroid extract for several years and had no myotonia. Thomasen states that the electromyogram was not typical of myotonia.

Our purpose in publishing this paper is to call attention to the fact that very severe myotonia may occur during states of thyroid deficiency and may be promptly relieved by adequate doses of thyroid extract.

CASE HISTORY NO. 1

A woman of 40 years developed chronic thyroiditis. Thyroid deficiency appeared and mild myotonia. The gland was removed completely and the patient soon developed extremely severe myotonia which made it impossible for her to work. Prompt recovery followed the administration of thyroid extract.

L. M., a white woman was seen for the first time in December 1949 with complaints of nervousness, palpitation, increased frequency of bowel movements and loss of 15 lbs. of weight. There were pea-sized adenomata in the upper poles of the thyroid gland but no clinical signs of hyperthyroidism. The basal metabolic rate was plus 14 per cent. Operation was advised but refused.

The patient was not seen again until March 1952. She stated that during the preceding months, she had gained weight and her thyroid gland had become larger and firmer. For a month she had noticed that her muscles were growing stiff. After sitting a while she found it very difficult to get up but after taking a few steps, she could walk with more facility. If she grasped an object she found it difficult to let go. There was no history of myotonia or of any muscular disorders in her family and the patient had never had such symptoms in the past.

On examination the skin was dry but the hair and nails normal. There was no edema of the eyelids. The thyroid gland was enlarged and very firm. Each lobe was as large as a small lemon. The basal metabolic rate was −7 per cent. The blood cholesterol was 425 mgs. per cent. Myotonia was strongly evident in the grips and was easily elicited by percussion of the muscles. The attempt to get out of a chair induced generalized myotonia.

On April 14, 1952 the thyroid gland was removed completely because of the possibility of carcinoma. Chronic thyroiditis, or Hashimoto's struma, was found, however. Three days later, one quarter grain of thyroid extract was given twice a day but there was no change in the muscular disorders. Calcium

did not help. The thyroid extract was discontinued and quinine was given in doses of 5 grains three times a day. This reduced the myotonia but did not abolish it. She was discharged from the hospital on April 21, 1952.

On May 7, the quinine was discontinued and the myotonia became so severe the patient was nearly helpless. Examination at that time revealed great difficulty in getting out of a chair and in taking the first step after being still for a time. After walking a short distance, she could walk with proper facility. The grips displayed strong myotonia. Percussion of the thenar muscles and the long extensors of the forearms produced a prolonged contraction. The muscles were not enlarged but seemed too firm for a woman of the patient's age. None of the standard signs of myotonic dystrophy were found. She seemed to be dull and her mental reactions were slow. Her face was puffy and her skin cold and dry. Otherwise the neurological examination was quite negative. There were no signs of latent tetany.

On May 17, the patient was placed on thyroid extract, grains one-half three times a day. Within two days after this medication was started, she showed great improvement in the muscular symptoms. On May 21, the dose was increased to two grains a day and on this dosage the patient was soon completely free of symptoms and myotonia could not be demonstrated on examination. The patient was able to return to her work. The changes in the skin disappeared and her mental conditon improved.

To the present time, i.e. February 1955, the patient has been maintained on one and a half to two grains of thyroid extract a day. When she is taking one and a half grains there may be slight myotonia during cold weather. When she is on two grains a day she has no myotonia whatever, but may have palpitation at times. It is evident that the relief of myotonia in this case requires full doses of thyroid extract. Small doses give partial relief and the symptoms begin to return within two weeks after the dose is reduced.

CASE HISTORY NO. 2*

A woman of 48 years had subtotal thyroidectomy because a cyst was causing pressure on the recurrens nerve. A few weeks later, she developed evidences of thyroid deficiency and extremely severe myotonia. The administration of thyroid extract abolished her symptoms within a short time.

Mrs. R. M., a woman of 48 years, developed a mass in the neck and was told by her physician that this was a cyst in the thyroid gland. At the end of the day her voice would get tired and hoarse and she was advised to have the mass removed. Subtotal thyroidectomy was performed in April of 1954. There were no apparent ill effects until some three or four weeks later. At that time she noticed that if she grasped an object she might be unable to relax her grip

* This patient was seen in consultation with Dr. W. H. Woody and later with Dr. H. M. Thomas, Jr.

promptly. It became increasingly difficult for her to get out of a chair. When she started to walk, she would get rigid all over and could scarcely move. After a few steps, however, she could walk freely. On several occasions when riding on a bus, she could not get out of her seat and had to be dragged out by other passengers. Once she sneezed and her entire body, including the muscles of respiration, became rigid so that she could not breathe for a short time. Ocular movements sometimes caused transient double vision. A sudden movement might cause a muscular cramp which was often painful. The patient stated that she would soon have to give up her position as a teacher for she could not go on. Calcium was administered without relief.

The patient firmly denied that she had ever had any muscular disorders before the operation. No history of any similar symptoms in the family could be elicited.

On examination on June 30, 1954, the patient's skin and hair were dry. Her face was puffy and the lower eyelids were swollen. Her memory did not seem clear and her mental reactions were slow. The neurological examination was quite negative except for myotonia. This was found in the eyelids and in the muscles of the arms, hands and legs. Strong myotonia was found in the grips. Percussion of the muscles of the extremities caused a prolonged contraction. The muscles were not altered in bulk or consistency. Evidences of myotonic dystrophy were not found. There were no signs of latent tetany.

The patient's blood cholesterol was found to be over 500 mgs. She was given thyroid extract and the dosage was rapidly increased to one and a half grains a day. There was improvement within a week. All the symptoms disappeared.

On January 15, 1955 the patient was re-examined. She stated that she was taking one and a half grains thyroid extract a day and that since starting this medication she had no motor difficulties whatever. Two grains a day were apt to make her heart beat too hard. Examination revealed no generalized myotonia or grip myotonia but a myotonic reaction to percussion was still evident in the long extensors of the forearms. The puffiness of the face and dryness of the skin had disappeared. She was no longer dull or slow in her reactions. The cholesterol was 258 mgs. per cent.

DISCUSSION

It is unfortunate that no electrical tests and no electromyograms were made. It is impossible to say whether the electromyogram, if made, would have showed the same changes found in the familial myotonias or not. There is no doubt, however, that these patients showed all the clinical evidences of myotonia such as is found in Thomsen's disease and myotonic dystrophy. We have never seen myotonia of such severity. The first patient was forced to stop work and the second, a school teacher whose symptoms reached their peak during

the summer vacation, would have been forced to resign in the fall, had she not responded to medication. In the first patient the myotonia was increased by cold and reduced by quinine just as one would expect if she had been suffering from one of the familial myotonias. The second patient's symptoms were abolished before the weather grew cold and she was never given quinine. The complete relief of the myotonia by the administration of thyroid extract, would seem to differentiate it clearly from that type found in the familial myotonias in which thyroid extract is not effective.*

It is quite clear that both patients were suffering from thyroid deficiency. The first patient developed mild myotonic symptoms when chronic thyroiditis began to cause hypothyroidism and her symptoms became much more severe, when the gland was removed. The second patient had no symptoms until a subtotal thyroidectomy was performed and then developed the usual signs of thyroid deficiency as well as myotonia.

In each case the symptoms began to recede within a week after the thyroid extract was administered and the patient remained symptom free on a daily dosage of one and a half to two grains. The first patient would have mild myotonia in cold weather when she was taking one and a half grains but had no myotonia whatever under any circumstances when taking two grains a day. The second patient had percussion myotonia when she was on one and a half grains a day, but no symptoms or disability of any type.

We feel sure that our patients do not have Thomsen's disease or myotonic dystrophy, because the family histories are negative, there is no wasting of the muscles, none of the evidences of endocrine disorder which are found in myotonic dystrophy are present and most important of all, the patients got complete relief of symptoms when thyroid extract was given.

Occasionally one finds thyroid deficiency in a case of myotonic dystrophy, as in the cases reported by Stanbury, Goldsmith and Gillis(13). In such instances the presence of the standard signs of myotonic dystrophy and the failure of the myotonia to respond to thyroid extract should make a differential diagnosis possible.

Several theories have been advanced to explain the myotonia in states of thyroid deficiency, but none are satisfactory. The suggestion that it is due to cerebellar involvement may be dismissed at once. Marinesco (9) in 1919 suggested that it is due to reduced cellular activity and lowered temperature of the muscles. He stressed the fact that the myotonia is reduced by heat and increased by cold.

* Poncher (11, 12) states that in the case of an infant suffering from myotonia congenita, complete recovery resulted from the administration of thyroid extract. There is good reason to believe that this infant was suffering from thyroid deficiency, however. Thomasen and other authorities agree that thyroid extract does not influence the myotonia of Thomsen's disease or myotonic dystrophy. Our experience leads us to agree with Thomasen.

CONCLUSIONS

Two cases are reported in which severe and incapacitating myotonia developed during states of thyroid deficiency. In each case, the symptoms were relieved completely by the administration of thyroid extract.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

April 4, 1955

CLINICAL IMPLICATIONS OF PHYSIOLOGICAL STUDIES IN OBSTRUCTIVE PULMONARY DISEASE

R. H. Shepard and R. L. Riley

A study of the relationship between the severity of obstruction of the airways and the extent of damage to the diffusing surface of the lungs has been made in 26 patients exhibiting the classical clinical picture of exertional dyspnea, expiratory obstruction, and hyperinflation of the chest.

Studies of lung volumes and of the maximum breathing capacity were used to estimate the severity of distention and airway obstruction. The maximal diffusing capacity was used to estimate the extent of damage to the diffusing surface of the lungs.

This lack of correlation suggests that long continued airway obstruction and overdistention of the lungs need not lead inevitably to damage to the walls of the alveoli. It may help to explain the discrepancy between ante-mortem and post-mortem findings in patients with emphysema since the former are based primarily on the severity of airway obstruction and the latter on the extent of damage to the alveolar walls.

The evaluation of ventilatory function, which can ordinarily be accomplished by functionally oriented clinical examination, provides an adequate basis for management of patients with emphysema since the consequences of airway obstruction dominate the clinical picture.

ANGIOCARDIOGRAPHY IN ATRIAL SEPTAL DEFECTS AND ANOMALIES OF THE PULMONARY VENOUS RETURN

John G. McAfee

In the past two years, intravenous angiocardiography has been used in attempting to differentiate isolated atrial septal defects from anomalous return of the pulmonary veins.

Twenty-four uncomplicated atrial defects, diagnosed on the basis of clinical examination and cardiac catheterization, have been studied by angiocardiography. The diagnosis was proven by surgery in six of these cases, and by post-mortem examination in one case. In only one-half of this group was the defect directly demonstrated by early opacification of the left auricle from the right auricle in lateral films. The atrial septal defects of the ostium primum type could not be demonstrated. Seventy percent of ostium secundum defects were demonstrated when the films were exposed at the rate of 3 to 4 per second. No false positive angiocardiographic diagnoses have been encountered.

Thirty-two cases of anomalous pulmonary venous return have been studied by angiocardiography, but the diagnosis was proven by surgery or post-mortem in only 12. The procedure demonstrated the anomaly completely in one-third of the cases and showed only part of the anomaly in another one-third. Eleven aberrant pulmonary veins which were revealed by the method cardiac catheterization were missed on angiocardiography, but in an equal number angiocardiography showed anomalous veins not demonstrated by catheterization. Anomalous insertions of the right pulmonary veins into the venae cavae or right atrium were much better demonstrated than those on the left side, because the left pulmonary veins were

obscured by overlying cardiac chambers. Total anomalous pulmonary venous return to the left innominate vein was readily demonstrated.

It is recommended that angiocardiography be carried out in addition to cardiac catheterization, in all cases of atrial septal defect or anomalous venous return prior to their surgical correction. Information can sometimes be obtained which can not be revealed by any other pre-operative diagnostic procedure.

EFFECT OF THYROXINE ON THYROIDAL RADIOIODINE ACCUMULATION

NORMAN E. SHARRER AND SAMUEL P. ASPER, JR.

The effect of thyroxine on the radioiodine uptake by the thyroid gland was studied in thirty-three euthyroid human subjects. The total quantity of thyroxine employed was administered in a single injection by the intravenous route to each subject, and radioiodine uptake by the thyroid was measured at intervals up to fourteen days after injection. In four of ten subjects, each administered an amount of *dl*-thyroxine varying between 0.75 mg. and 4.0 mg. during the course of a radioiodine uptake test, a transient inhibition of thyroidal accumulation appeared within one and one-half hours. This effect persisted no longer than one and one-half hours, following which uptake resumed at the original rate. Six subjects showed little or no inhibitory effect. The physiologic significance of this result is not clear.

Radioiodine uptake by the thyroid gland was determined at intervals between four hours and fourteen days after thyroxine injection in twenty-three subjects. Seventeen subjects received 3.0 mg. of *dl*-thyroxine, 5 subjects 3.0 mg. *l*-thyroxine, and one subject 1.5 mg. *dl*-thyroxine. In two subjects no diminution in uptake was observed when studied at 4 days and 7 days. In twenty-one subjects, a decrease in thyroidal accumulation was observed which was variable at one day, marked at four days and maximal at 7 days. A comparison of the time relationships of this inhibitory effect with that reported to occur after hypophysectomy in rats, revealed striking similarity. This is believed to demonstrate that in euthyroid subjects, the major mechanism of thyroxine-induced suppression of thyroidal radioiodine uptake is inhibition of thyrotropin release from the hypophysis.

CITATION FOR DR. SAMUEL JAMES CROWE WHEN HE WAS AWARDED THE DEGREE OF DOCTOR OF SCIENCE BY THE JOHNS HOPKINS UNIVERSITY ON FEBRUARY 22, 1955

EDWARDS A. PARK

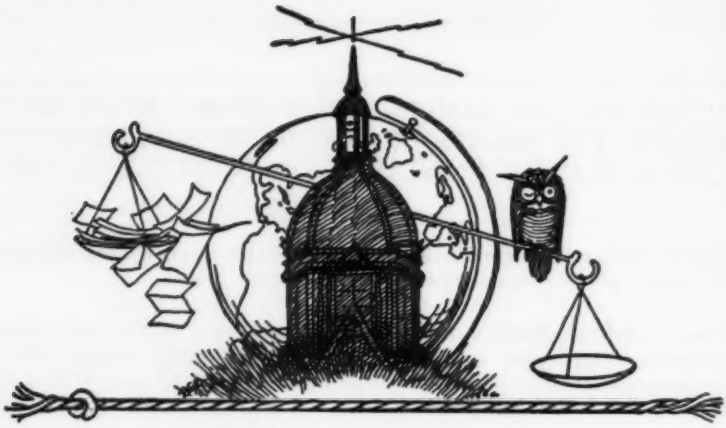
On behalf of the Faculties of The University and with the approval of the Board of Trustees, I have the honor to present a candidate for the degree of Doctor of Science.

Samuel James Crowe, Virginian, son of a physician, graduate of the University of Georgia, bent on becoming an engineer but persuaded by his father to study medicine; faltering in allegiance to medicine during his first year at Johns Hopkins until by accident encountering Professor William Halsted in a peculiarly personal way; then overnight falling under the spell of that great man and transformed into his ardent disciple. Intern, assistant resident, research student for five years, ushered into investigation through the doorway of surgery under that dynamic perfectionist in technique, Harvey Cushing; planning on a career in brain surgery with him at Harvard, but in a dramatic moment suddenly invited by Dr. Halsted to alter the course of his life by accepting the directorship of the newly planned division of otolaryngology, a subject about which he knew nothing. Bewildered but accepting, he began his independent career at the age of 29.

Among his far flung accomplishments which have extended even to studies on the physiology of the adrenal gland and hypophysis, these:—The creation of the first great clinic of otolaryngology in this country, conceived in the grand perspective of general surgery, pervaded by the spirit and example of its head, an oasis in a desert of medical practice; The first penetration into the terra incognita of deafness in the human being, coincident with the founding of the Otological Research Laboratory, accomplished through painstaking correlations between specific defects in hearing and the corresponding anatomical defects in the organ of Corti of the inner ear; The first to recognize as a major cause of deafness in children lymphoid ingrowths about the eustachian tubes, the development by him of a method for prevention and treatment through radium, now widely adopted and organized throughout this country and in Europe which has safeguarded the hearing of countless persons.

Taciturn, shy, with heart of gold, unconscious of himself in his consecretion to ideals, skilled surgeon, foremost explorer and acknowledged intellectual leader in his subject, teacher of teachers, in his inner demand for perfection in the mold of his great master, the life and light of his department, a constant joy to his friends, bulwark to his colleagues and pride to the Medical School as one of its great sons, in honoring whom this University is but doing homage to itself.

I am honored, Mr. President, to present Samuel James Crowe, Professor Emeritus of Laryngology and Otology of The Johns Hopkins University for the degree of Doctor of Science.



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Lehrbuch der Auskultation und Perkussion. By K. HOLLDACK, with a foreword by CURT OEHME. 195 pp., Ganzleinen DM 27. ($6.40). Georg Thieme Verlag, Stuttgart, Germany. Also Intercontinental Medical Book Corp., New York, N. Y.

This book is inaccurately titled. It is in fact a detailed treatise on physical diagnosis and provides instruction in the performance of the entire systematic and thorough physical examination, excluding specialized aspects such as the neurological examination. It is written for the medical student or at least will prove most useful to this group. It is, however, an authoritative discussion of the subject presented in detailed yet succinct manner. In general this is truly an excellent textbook of physical diagnosis, as good or possibly better than any available in English.

It will, of course, not be useful to American students because of the linguistic barrier. However, it should be useful as a guide to those who are revising (or writing *de novo*) textbooks of physical diagnosis. No bibliographic references are provided and the book will, therefore, be of limited usefulness to an investigator using phonocardiographic techniques, for example.

Wherein lies its excellence? Its organization is conventional and follows the sequence used in performance of the physical examination. In the first place, the book is good because its author is a thoroughly competent and well-rounded physician whom I came to respect from contact with him on his visits to this country in 1951 and 1954. In the second place, the art and science of percussion and auscultation is presented as a dynamic field and not as the dead subject it is sometimes considered. Holldack is particularly suited to present the subject material from this vital point of view since he has been a leader in phonocardiographic investigation in recent years. His monograph on the subject in *Ergebnisse der Inneren Medizin und Kinderheilkunde* for 1952 is one of the best recent reviews. He is a prominent member of the fraternity of phonocardiographers which includes, as a very incomplete enumeration, Mannheimer in Sweden, Lian and Gallavardin in France, Calo in Tunis, Evans and Leatham in London, Rytand, Wolferth and Wood, Dock, Sprague and Rappaport in this country, Riviero Cavallo in Mexico, Orias, Braun-Menendez, and Cossio in Argentina.

In the third place, the excellence of the book resides in part in the illustrative material.

Very graphic sketches, line drawings for the most part, illustrate features such as body types, facies typical of various disease states, manual techniques in the physical examination, areas of maximum audibility of murmurs, and so on. Those features of the physical examination in which sound is concerned—and acoustic phenomena constitutes such a large portion of the physical examination—are profusely illustrated with oscillographic recordings of the sound or idealized line drawings of same. These are very helpful. I would predict, however, that in a few years spectrograms (e.g. spectral phonocardiograms) of these sounds will replace oscillograms in textbooks of physical diagnosis. The spectrograms will make it much easier to convey to the student precisely how these sounds impress the ear.

VICTOR A. MCKUSICK

Groves' Synopsis of Surgery, 14th edition. Edited by SIR CECIL WAKELEY. 651 pp., $7.00. The Williams & Wilkins Co., Baltimore, Md.

A pocket-sized 650 page volume, this book is the fourteenth edition of Hey Groves' Synopsis of Surgery, originally published in 1908. It attempts to reduce basic surgical knowledge to outline form for quick review or reference. It is directed toward the student and to the practitioner who is unable to keep abreast of developments in the many surgical fields.

It is obviously a difficult task to summarize the field of surgery. This book is a creditable job. The information on any given subject is necessarily limited and dogmatic but the information provided is usually an adequate survey of the subject matter.

This volume may be of use to students for review purposes or to general practitioners who require only a very limited amount of information on a given surgical topic.

JAMES R. CANTRELL

Essentials of Medicine, 17th edition. By CHARLES PHILLIPS EMERSON, JR. and JANE SHERBURN BRAGDON. 922 pp., $4.75. J. B. Lippincott Co., Philadelphia, Pa.

This is a work of considerable merit. Although designed particularly for the guidance of student nurses, much of the content exceeds the scope of the student level and is worthy of the attention of the graduate nurse and instructor as well. Especially commendable features include the following: analysis of disease states according to physiologic systems in a thorough and logical pattern; excellent introductory discussions of normal physiology and concluding summaries of practical information in each chapter devoted to important body systems; and a remarkable degree of modernization demonstrated by the up-to-date content on treatment, pharmacology and bibliography. To the student nurse certain limitations will appear. Discussions of the more common disease states encountered in clinical training are generally accorded no more detail than the infrequent and rare syndromes. The lists of selected references are largely derived from physicians' journals which are likely to be too advanced for the level of the average student. Detailed instructions to guide the student in nursing procedures are extremely limited. The theoretic and physiologic considerations, on the other hand, are presented very clearly and should enable the capable student nurse to acquire an excellent background in medical nursing. The format, paper quality, and binding are in keeping with the superior quality of the content of this volume.

N. E. S.

The Concept of Schizophrenia. By W. F. MCAULEY. With a Foreword by JOHN H. EWEN. 145 pp., $3.75. Philosophical Library, New York, N. Y.

This small book is a valuable review of the knowledge accumulated concerning schizophrenia. In simple terms, the development of the concept is delineated with special reference to the contributions of Kraepelin, Bleuler, Meyer and their contemporaries. This is followed

by consideration of aspects that have been studied with increasing detail and are of growing interest, namely, heredity, social and environmental factors, neurophysiology and metabolism. At the end there is a brief section on diagnosis and treatment. The bibliography is compact.

References to the psychoanalytic concepts regarding schizophrenia might have been more clearly assembled; they are mostly dispersed throughout the presentation, and in some respects are not complete. Also, the hereditary aspects are not presented in as sharp a focus as could be done. But in a way this should provoke additional interest rather than prove disappointing.

This book should be valuable not only to the student who wishes to acquaint himself with the clinical picture of schizophrenia and the historical setting of research in this field, but also to the connoisseur of the disease, who may be led to appreciate more fully the variety of facts and theories so far considered and perhaps be stimulated to add new perspectives to his or her understanding of this disease.

EDWARD L. SUAREZ-MURIAS

Handbook of Radiology. Edited by RUSSELL H. MORGAN. Associate Editor, KENNETH E. CORRIGAN. 518 pp., $10.00. Year Book Publishers, Chicago, Illinois.

A wealth of information has been assembled in this compact volume, and its organization permits ready access. Designed as a tool for daily use, the radiation worker will find it of increasing value as he becomes familiar with its contents. Wherever possible the presentation is in tabular or diagrammatic form with a minimum of explanatory material. The print is good and the tables and drawings are of excellent quality. Those limited to either diagnosis or therapy may naturally feel that the other discipline has received too much attention, but within the limitations of space the book seems very complete.

The first two sections deal with basic definitions of physical terms and a comprehensive collection of tables for the elements and their properties, conversion factors, atomic attenuation coefficients, and others. Mathematical tables and diagrams for various radiation generators are placed at the back of the book. Depth dose data for X-ray and Cobalt 60 are given under radiotherapy along with the rules and tables for both the Quimby and Paterson-Parker systems of radium dosimetry. Data on radioactive sources other than radium are also given. A separate section is devoted to radio-isotopes and over twenty of the frequently used elements are discussed.

Radiographic materials from X-ray tubes to processing agents are described thoroughly and brief accounts are given of all the contrast media in current use. In describing thoracic aortography, Diodrast and Urokon 70 per cent are suggested. However, it is generally accepted now that concentrations over 35 per cent are likely to cause brain damage and should never be used. Line drawings for radiographic positioning are very clear and are accompanied by suitable technic charts. New tables are introduced for the computation of protection barriers against a wide range of radiation generators. A particularly valuable chart permits the calculation of approximate dosage in roentgens for diagnostic exposures.

FRED E. STULL, JR.

The Atom Story. By J. G. FEINBERG. Foreword by Prof. F. SODDY. 243 pp., $4.75. Philosophical Library, New York, N. Y.

A British scientist garners here for us the lore of the atom, developing with gathering impetus the tale of its structure and that of the nucleus from Democritus to the hydrogen bomb. With engaging simplicity he brings out the part of each chemist and physicist, enlivening the plodding progress with humor, anecdotes and homely illustrations, without

omitting that keen eye for detail which guided the quest. The drama of the years from '40 to '45 with disclosure of the uranium isotopes, their production and control, leads straight to the denouement: the dilemma of nuclear energy for peace or war.

Demanding no special knowledge, but infused with an enthusiasm and glow of language to beguile the late evening hour, this small book delivers that rare synthesis, a dense package of information which is also a pleasure to read.

S. A. T.

Peripheral Nerve Injuries. Medical Research Council Special Report Series No. 282. By the Nerve Injuries Committee of the Medical Research Council. Edited by H. J. SEDDON. 451 pp., £2 15s. Od. net. Her Majesty's Stationery Office, London, England.

This book is a compilation of a series of studies made under the direction of a Nerve Injuries Committee appointed by the British Medical Research Council's Committee upon Injuries of the Nervous System. The establishment, by the Ministry of Health in Britain, of five Nerve Injury Centers during the Second World War resulted in stable hospital conditions for effective investigation of nerve injuries, and presented unified facilities for the re-examination of patients. A report by a similar committee following World War I was devoted principally to the clinical aspects of nerve injury, the phenomena of recovery, etc.

The present report makes no attempt to present an organized clinical account of the diagnosis and treatment of nerve injuries, but is concerned only with "those advances in the understanding of nerve injuries made by British workers during the war years." Using the approach of the investigator, the book presents essays on many aspects of injury to the peripheral nerves, such as rates of regeneration, vasomotor and nutritional disturbances following nerve injury, as well as histopathological studies. There are chapters on electrical diagnosis and electromyography. Significant analyses are made of the results of nerve suture and grafting, while a long study of the factors influencing functional recovery gives a practical slant to the work. The book will be useful to the investigator of every aspect of peripheral nerve injuries but is not at all intended as a practical handbook for the surgeon.

F. J. O.

Organic Chemistry. An Advanced Treatise. Vols. III and IV. Edited by HENRY GILMAN. 1245 pp., $8.75 each. John Wiley & Sons, New York, N. Y.

These two volumes, like the first two published in 1943, are important contributions to the continued education of the chemist in the fields of organic and biological chemistry. Together with the first two volumes they comprise a body of well-organized and probably soundly evaluated knowledge so directed as to provide penetration into many chemical subjects that are continually and elusively expanding.

The material concerns major concepts in the various fields and is developed in a concrete fashion through specific detail well-documented with selected, more recent references. The emphasis is not on review, however, and so the chapters are not complete in this sense. Rather the emphasis is on more recent developments, but the work avoids the high specialization in some one aspect of a subject reserved usually for monographs.

The investigator in medical and biological fields should find that the volumes fill a special and frequent need for some critical grasp of pertinent subject matter, so hard to obtain from either monographs or reviews. The same opportunity is available to the physician who, after preliminary orientation and instruction, wishes to inquire more fully about the background of clinical phenomena. The books are more than textbooks or reference books in that they repay frequent, thoughtful consultation.

The format provides ease of accessibility in size of print, organization, large index, extensive tables of contents for each chapter, and cross-references. The subjects covered by the third volume are: "The Study of Organic Reaction Mechanisms", "Applications of Infrared and Ultraviolet Spectra to Organic Chemistry", "Lipids", "Organic Dyes", "Some Aspects of Chemotherapy", and "Antibiotics". In volume four, these chapters are included: "The Terpenes", "Heterocyclic Chemistry", "Starch", "Chemistry of Explosives", "Reactions of Organic Gases Under Pressure", and "Oxidation Processes". The editorial board consists of outstanding chemists who give a high degree of authority to the work. The reviewer cannot present further comment on each chapter because his specific knowledge of each subject is not sufficiently adequate.

FREDERICK W. BARNES, JR.

Veterans Administration Technical Bulletins, Series 10, Vol. VI, 1952 and 1953. Edited by ARTHUR M. WALKER. 44 pp. U. S. Government Printing Office.

This is a compilation of the weekly bulletins issued by the Veterans Administration for its Medical Staff during the years 1952 and 1953. The original purpose of the bulletins first issued in 1946 was "to inform doctors returning from World War II, who had necessarily been out of touch with medical progress, of the events which had occurred during their absence and upon problems arising from the war." Certainly these bulletins have assumed a broader role now. They are reviews upon specific subjects, and, indeed, this volume contains many concise, well written and informative reviews of timely interest to the clinician. Each review is written by a well-recognized authority in the field. Such subjects as malignancy in the colon, pneumonia, gout, and treatment of thyrotoxicosis are presented by such authorities as Drs. J. E. Roads, Maxwell Finland, John Talbott, and J. H. Means, respectively. The references are many. The editing has been very well done and the indexing is correct. Included in this volume are reprints of two medical classics (in this reviewer's opinion) which should be read and re-read by every clinician—the one—Dr. Alan Gregg's AOA lecture on scholarship "Emergent Ability," and the other, Dr. Francis Peabody's lecture on the philosophy of medical practice, "The Care of the Patient." The inclusion of these two articles certainly enhances the value of this volume. The printing of both of these articles together in a single volume makes personal ownership of this volume highly desirable.

JOHN COLLINS HARVEY

BOOKS RECEIVED FOR REVIEW

Analytical Cytology. Newer Methods for Studying Cellular Form and Function. Edited by ROBERT C. MELLORS. Foreword by FRANCIS O. SCHMITT. 512 pp., $15.00. The Blakiston Division, McGraw-Hill Book Co., New York, N. Y.

Blutkrankheiten. Methodik, Klinik, Therapie. By HANNS FLEISCHHACKER. 247 pp., $7.50. Wilhelm Maudrich, Publisher, Vienna, Austria. Also Medical Book Corp., New York. N. Y.

Clinical Bacteriology. By E. JOAN STOKES. Foreword by A. A. MILES. 288 pp., $5.00. Edward Arnold, Publishers, London, England. Williams & Wilkins Co., Baltimore, Md.

Cold Injury. Transactions of the Third Conference February 22, 23, 24 and 25, 1954. Edited by M. IRENE FERRER. 226 pp., $4.50. The Josiah Macy, Jr. Foundation, New York. N. Y.

Cytologie des Weiblichen Genitalkarzinomas. (1st zubleich Wiener Beiträge zur Gynäkologie, Band 2). By EDMUND SCHÜLLER. 129 pp., $6.00. Wilhelm Maudrich, Publisher, Vienna, Austria. Also Intercontinental Medical Book Corp., New York, N. Y.

Differential Diagnosis of Internal Diseases. Clinical Analysis and Synthesis of Symptoms and Signs on Pathophysiologic Basis, 2nd revised and enlarged edition. By JULIUS BAUER. 987 pp., $15.00. Grune & Stratton, New York, N. Y.

Gestation. Transactions of the First Conference March 9, 10 and 11, 1954. Edited by LOUIS B. FLEXNER. 238 pp., $5.00. The Josiah Macy, Jr. Foundation, New York, N. Y.

Letalfaktoren. In Ihrer Bedeutung für Erbpathologie und Genphysiologie der Entwicklung. By ERNST HADORN. 338 pp., Ganzleinen DM 39. ($9.30). Georg Thieme Verlag, Stuttgart, Germany. Also Intercontinental Medical Corp., New York, N. Y.

Management of Addictions. Edited by EDWARD PODOLSKY. 413 pp., $7.50. Philosophical Library, New York, N. Y.

Medical Progress 1955. A Review of Medical Advances During 1954. Edited by MORRIS FISHBEIN. 346 pp., $5.00. McGraw-Hill Book Co., New York, N. Y.

Practical Endocrinology. By LEWIS M. HURXTHAL in cooperation with A. SEYMOUR PARKER and HIRSH SULKOWITCH. 318 pp., $7.00. The Blakiston Division, McGraw-Hill Book Co., New York, N. Y.

The Skin. A Clinicopathologic Treatise. By ARTHUR C. ALLEN. 1048 pp., $25.00. The C. V. Mosby Co., St. Louis, Missouri.

Stress Situations. Edited by SAMUEL LIEBMAN. 144 pp., $3.00. J. B. Lippincott Co., Philadelphia, Pa.

Tumor-Host Studies. The Physiological and Pharmacological Action of an Iodide-trapping Substance Formed in Tumor-bearing Animals. By KENNETH G. SCOTT and CHIN-TZU PENG. (University of California Publications in Pharmacology, Vol. 2, No. 22, pp. 345–376, plates 27–31, 2 figures in text.) 75 cents. University of California Press, Berkeley, California.